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CONTINUAL CORNIFIED VAGINAL CELLS AS AN INDEX OF AVITAMINOSIS-A IN RATS*

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THE reliability of continual cornified vaginal cells in rats as a symptom of vitamin A deficiency has been the subject of several conflicting reports. Evans (3, 5), Macy, Outhouse, Long and Graham (11) and Hohlweg and Dohrn (7) consider the presence of cornified cells in the epithelial layers of the vagina to be the first and most delicate symptom of vitamin A exhaustion. On the other hand, Parkes and Drummond (14), and Coward *et al* (1, 2) conclude that continual cornified cells in the vaginas of rats are not a reliable index of A deficiency.

The material reported in this paper, collected in the course of experiments with rats on diets low in vitamin A, was studied to see, first, in what per cent of instances continual cornified cells in the vagina were associated with vitamin A depletion; secondly, to determine whether or not continual cornified vaginal cells were a more delicate gauge of the depletion of vitamin A than other symptoms; thirdly, to find whether there was any constant time-relation between daily cornified cells in the vagina and other symptoms of avitaminosis.

The stock from which most of the rats for this experiment were obtained came from animals reared by Helen Dean King at the Wistar Institute (10). They represent the 66th to the 68th generation of brother and sister mating. Of forty control animals, the modal age of the opening of the vagina was 36 to 39 days. The mean was 37 days with an S. D. of 3.30. Having the vaginas open at this time precluded any necessity of artificially rupturing the membrane, and allowed the smear to be watched soon after the rats were put on the experimental diet. One or more normal cycles could be observed before the effects of the diet became apparent.

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The rats in Group IV, Tables II and III came from the Connecticut Agricultural Experiment Station.

The care and feeding of the stock animals from which the experimental rats were obtained were standardized as much as possible. Each pair of rats was kept in a separate cage. Strips of paper or cotton were used for bedding, which was changed once or twice a week. Trays, water bottles and tubes were washed and sterilized with like frequency. Stock animals were fed two types of diet. One group was fed 100 per cent calf meal, the other group was fed table scraps. Calf-meal is a commercial mixture containing approximately the following ingredients in 100 parts; oil meal 15, malted barley 10, whole wheat 22, oat flour 15, dried skim milk 15, yellow corn meal 20, ground limestone 1, steamed bone meal 1, salt 1. It is low in vitamin A. The table scraps include carrots, greens, cereals, milk, and meat, but no additional A is given in the form of cod liver oil.

Breeding females were mated at about one hundred days of age. All litters were reduced to six on the day of parturition.

Each experimental rat was isolated in a wire mesh cage. A wire base allowed feces to drop through. Partitions were kept between cages to prevent the spread of infection. The water bottles and tubes were washed and sterilized twice a week; cages were sterilized weekly. Rubber gloves were worn and were dipped in lysol after each animal had been handled. The animals were weighed twice a week. Once a week residual food was weighed and thrown out, the container sterilized and fresh food given. The weight of the spilled food was recorded in order that food-consumption might be determined. The animals were observed every day. If the food containers became empty at any time, supplementary food was given and recorded.

Vitamins B, D, and E were supplied by yeast, irradiation and hydrogenated vegetable fat.[1] Yeast[2] or irradiated ergosterol or a mixture of both was fed either four or six times a week in separate sterilized containers. One tenth of a gram daily of tested yeast was sufficient to produce a gain of weight in rats depleted of vitamin B. Two to eight tenths of a gram daily was fed to these experimental animals. Vitamin D was supplied by irradiation. The yeast, spread in thin layers, was exposed to the rays of a mercury vapor lamp at a distance of 15 inches for 15 minutes, then mixed, respread and irradiated for another 15 minutes (Hess 6).

Vitamin E was contained in the hydrogenated vegetable fat. Kennedy

[1] Crisco.

[2] Tested yeast supplied by M. H. Givens of the Northwestern Yeast Co.

and Palmer (9) secured successful reproduction in rats on a purified diet containing 15 per cent of the fat, whereas replacement of the latter with 15 per cent of lard, resulted in sterility. Evans and Burr (4) secured "cures" in sterile rats, on E-free diets, by introducing 22 per cent of hydrogenated vegetable fat in the diet or by feeding daily supplements of 500 mg. of it during the gestation period.

The room temperature was adjusted between 80 and 84 degrees Fahrenheit. In this way it was possible to keep the rats alive for longer periods after severe symptoms of avitaminosis had developed.

Examination of the vaginal orifice was made every second or third day. After the vagina opened, smears were taken four to five times a week until the smears remained cornified for about ten days. After that, smears were taken five to six times a week on 16 animals, four times a week on 9 animals, and from two to three times a week on the remaining rats. The proportion and type of cells were recorded at each examination.

Smears were taken by the lavage method using tap water colored with neutral red. Small pipettes were made for this purpose. They were rinsed with mercuric chloride and then with water, or a fresh pipette was used for each animal in order to prevent infection.

A total of 101 female rats was used. Eight rats received Diets A or B shown in Table I. Ninety-three animals were fed diet No. 1 in Table I. Forty-one died or were killed before the vagina opened. All rats were killed immediately on the appearance of pneumonia. Records of the remaining 52 and the 8 rats on diets A and B are shown in Tables II and III.

The first group shown in Tables II and III was given extracted casein prepared according to the method given by Jackson (8). The second group was fed the technical casein without extraction. The third group, consisting of rats whose mothers were fed 100 per cent calf-meal diet, was given technical casein. The ingredients in the diet are in the proportions given in Table I.

All the rats in Groups I, II and III were weaned and placed upon the experimental diets at 21 to 24 days of age. At this time Groups I and II weighed approximately 39 grams and Group III weighed on the average ten grams less (Table III).

The average time in days at which the vaginas opened for the three groups (Table II) is 38.9 days (S.D. = 7.1), 39.9 days (S.D. = 6.2), 37.4 days (S.D. = 6.9). The corresponding standard deviations of the means (S.D.M.) 1.9; 1.3; 1.8 show that the time of the opening of the vaginas in each group was essentially the same. The weight of the animals at this time

averaged over eighty grams for Groups I and II, Table III, (85.2 gm. S.D. = 12.5; 80.3 gm. S.D. = 10.9) and more than 20 grams less for Group III (56.7 gm. S.D. = 12.6).

The average time at which the rats show the continual cornified smear is about the same for the group wherein mothers were on table-scrap diet, with progeny on a diet including extracted casein, Table II, Group I, (43.4 days S.D. = 5.8) and for the group where parents were on calf-meal and progeny were fed technical casein (46.4 days S.D. = 4.6) Group III. Group II from mothers on a table-scrap diet fed technical casein showed continual cornified cells over two weeks later (60.9 days S.D. = 7.8). In Group I they probably started with a storage of the vitamin; in Group III the storage of vitamin was less, but the technical casein contained traces. The rats in Group II started with a storage of vitamin A and also had traces in their diet. This group had continual cornified cells considerably later. When the two groups bred from mothers on a table-scrap diet are compared (Groups I and II) the animals fed extracted casein show continual cornified cells over two weeks earlier than the rats fed technical casein. The weights are roughly in proportion to what might be expected

TABLE I

PER CENT COMPOSITION OF VITAMIN A DEFICIENT DIETS

Ingredient	Diet No. 1.	Diet A	Diet B
Casein	15–18**	15	
Cornstarch	56–63**	22	
Hydrogenated vegetable fat*	18–22**		
Osborne and Mendel salt mixture IV	4	3	2
Lard		10	
Tobacco seed, ground		50	98
Yeast	0.4–0.8 gm. non-irradiated	0.2 gm. non-irradiated	0.2 gm. non-irradiated
Irradiated ergosterol	0.001–0.002 gm.	0.001 gm.	0.001 gm.

Note 1. The yeast and irradiated ergosterol were fed daily or every other day in a separate dish.

* Crisco.

** The rats ate more when the food was varied, so the diets were changed weekly or biweekly. The amounts of casein, cornstarch and Crisco were changed in the following proportions from 15, 63, 18, to 18, 56, 22 respectively.

Eighteen rats were weaned to a diet the same as above except that 0.8 gm. of yeast, half of which was irradiated, was given. In four instances no ergosterol was given until 6 to 9 days after weaning. In eleven instances no ergosterol was given until 21 to 28 days after weaning.

from the amount of stored and available vitamin A (Table III). Groups I and II average about one hundred grams (99.7 gm. S.D. = 10.4; 110.8 gm. S.D. = 15.1) and weigh on the average over twenty-four grams more than Group III (77.0 gm. S.D. = 14.6).

The failure to gain in weight or the appearance of xerophthalmia is considered pathognomonic of vitamin A deficiency. The average time for the appearance of xerophthalmia or these symptoms when weight-gain stopped shows a great difference in the three groups. Failure to gain in weight was recorded on the day that the weight did not show an increase over the last weight, and after which it remained the same or declined.

The first group of animals to show a loss of weight or xerophthalmia was composed of rats from mothers on a calf-meal diet fed technical casein, Group III, (58.7 days S.D. = 3.0). They weighed an average of 86.3 grams (S.D. = 17.1). The second group to show a loss of weight (Group I) consists of rats from mothers on a table-scrap diet fed extracted casein (70.3 days S.D. = 9.0) whose average weight was 123.8 grams (S.D. = 19.3). Last to show a second symptom of avitaminosis were the animals from mothers on a table-scrap diet fed technical casein (89.9 days S.D. = 14.0) whose weight averaged 126.3 grams (S.D. = 15.2). The animals to show the greatest amount of similarity within the group for the time in which xerophthalmia appeared or weight-gain stopped are those in Group III. Here the standard deviation of the mean is 0.8 as compared to approximately 3 for Groups I and II. This is to be expected because the parents were on controlled diets, low in vitamin A.

Seven rats died before the complete series of observations could be made; thus the number throughout the group is not constant. In four rats the vaginas did not open in the first forty days. In each instance when the vagina opened cornified cells were present and continued cornified. There was no way to tell how long they had been present; hence these animals could not be used in computing the interval in Table II. The interval was estimated on 48 animals by subtracting the age of the animal at the appearance of continual cornified cells in the vaginal smear, from the age of the animal when the second symptom of avitaminosis appeared or, as in Group IV, the time the experiment was terminated. These individual differences were averaged and the dispersion-measures computed directly.

The interval between the appearance of cornified cells in the vagina and the second symptom of avitaminosis is about the same for Groups I and II, Table II, (27.8 days S.D. = 9.0; 28.3 days S.D. = 15.0). In Group I, however, both symptoms appear at an earlier age than in Group II. The

interval between the first and second symptom of avitaminosis in Group III (12.5 days S.D. = 4.8) of animals from mothers on a calf-meal diet is less than half that of Group I or II of animals from mothers on table-scrap diets. The more rapidly the animal is depleted of vitamin A, the shorter the interval. It is interesting that even in this group the appearance of

TABLE II

AGE OF RATS ON VITAMIN A-DEFICIENT DIET
(age in days)

	Vagina opened	Continued cornified smear	Appearance of xerophthalmia or weight gain stopped	Experiment terminated	Interval*	
Group I:** rats on A-deficient diets made with extracted casein; mothers on table scrap diet.						
Number of rats	14	10	12		10	
Mean	38.9	43.4	70.3		27.8	Significant
S.D.	7.1	5.8	9.0		9.0	
S.D.M.	1.9	1.8	2.6		2.8	
Group II:** rats on A-deficient diets made with technical casein; mothers on table scrap diets						
Number of rats	24	20	19		19	
Mean	39.9	60.9	89.9		28.3	Significant
S.D.	6.2	7.8	14.0		15.0	
S.D.M.	1.3	1.7	3.2		3.4	
Group III:** rats on A-deficient diets made with technical casein; mothers on 100 per cent calf meal.						
Number of rats	14	12	14		12	
Mean	37.4	46.4	58.7		12.5	Significant
S.D.	6.9	4.6	3.0		4.8	
S.D.M.	1.8	1.3	0.8		1.4	
Group IV:*** rats on A-deficient diets made with tobacco seed.						
Number of rats	8	7	—	8	7	
Mean	76.0	111.3		169.4	69.9	Significant
S.D.	28.3	29.9		38.6	25.7	
S.D.M.	10.0	11.3		13.7	9.7	

* Interval represents the difference between the apperance of continued cornified smears and the appearance of xerophthalmia, or weight gain stopped, or as in Croup IV the time the experiment was terminated, of each rat subtracted separately then averaged.

** The rats were weaned and placed on the experimental diets at 21 to 24 days of age.

*** The rats were placed on the experimental diets at from 28 to 37 days of age.

continual cornified cells precedes other symptoms. It is conceivable that in rapidly depleted groups the two symptoms might appear simultaneously.

In order to see whether a group of animals could be maintained with a certain amount of vitamin A, such that cells of the vagina would become constantly cornified without the appearance of any other symptom of avitaminosis, the following experiment was done. Eight rats, divided into two groups of four each, were put on Diets A and B from 28 to 37 days of age (Table I). Their history is given in the fourth group of Tables II and III. The vaginas opened late compared to the inbred rats from the King stock which have an early maturity (mean 76.0 days S.D. = 28.3). Their weight at this time averaged 104.9 grams (S.D. = 18.3). Continual cornified cells did not appear until about 111.3 days (S.D. = 29.9) because the food contained some vitamin A. The weight averaged 141.7 grams (S.D. = 30.0). No other symptoms developed. When the animals weighed 150 grams, or over, there was some fluctuation in their daily weight. Unless an infection developed, there was no pronounced continuous drop in weight. In one instance the cells remained cornified for 126 days. Cornified cells might have remained for the entire normal life of the animal if the experiment had not been terminated in each instance by administering cod liver oil or by death. From twenty-four hours to three days after the dose of cod liver oil, continual cornified cells disappeared and the normal cycles were observed. Group IV cannot be compared directly with the other three groups. The rats did not come from the same parents and were not fed similar diets.

In the first three groups, however, the difference in time of the appearance of cornified cells and the second symptom of avitaminosis is statistically significant. In Group IV, by design, the animals were allowed to continue under fixed dietary conditions for an interval greatly in excess of that required for the other three groups. Since a second symptom of avitaminosis did not develop, the intervals between the appearance of cornified cells and the termination of the experiment were calculated and found to be statistically significant.

The first indication of a change in the oestrus cycle appeared as a lengthening of the oestrus and a shortening of the dioestrous interval. Gradually the cornified phase extended until it had become continuous. About the time the cells became cornified, the vagina seemed very dry (Manville, 13). The animals had to be handled carefully in order to prevent trauma to the mucosae of the vagina when taking the lavage. In spite of every pre-

caution some animals had necrosis and sloughing of the mucosae. This was shown in the smears by an excessive number of leucocytes, the presence of discrete red blood cells, or by hemorrhage.

In every rat where the smear had shown cornified cells for two weeks or longer, leucocytes in the vagina were reported at some time in its history.

TABLE III

WEIGHT OF RATS ON VITAMIN A-DEFICIENT DIET
(weight in grams)

Put on diet		Vagina opened	Continued cornified smear	Appearance of xerophthalmia or weight gain stopped
Group I: rats on A-deficient diets made with extracted casein; mothers on table scrap diet.				
Number of rats	14	14	10	12
Mean	39.3	85.2	99.7	123.8
S.D.	5.1	12.5	10.4	19.3
S.D.M.	1.4	3.3	3.3	5.6
Group II: rats on A-deficient diets made with technical casein; mothers on table scrap diet.				
Number of rats	24	24	20	19
Mean	37.8	80.3	110.8	126.3
S.D.	7.2	10.9	15.1	15.2
S.D.M.	1.5	2.2	3.4	3.5
Group III: rats on A-deficient diets made with technical casein; mothers on 100 per cent calf meal.				
Number of rats	14	14	12	14
Mean	28.3	56.7	77.0	86.3
S.D.	4.0	12.6	14.6	17.1
S.D.M.	1.0	3.4	4.2	4.6
Group IV: rats on A-deficient diets made with tobacco seed.				
Number of rats		8	7	
Mean		104.9	141.7	
S.D.		18.3	30.0	
S.D.M.		6.4	11.3	

The time when leucocytes are present is significant. In some rats on A-deficient diets they were present in only one smear during the period of constant cornification. In others they might be mingled with the cornified cells for weeks at a time. This is quite different from their rhythmic appearance in the normal animal. Out of a total of 506 smears taken after

the vagina had been constantly cornified, 119 (24 per cent) contained leucocytes.

The vaginas of 33 rats were sectioned for further study. In every instance cornified cells were seen at the periphery. Five rats showed necrosis of the mucosae. One had a recent hemorrhage with free blood in the lumen. Some of the animals had been on the A-deficient diet a long time before being killed, which would probably make the incidence of infection higher. In 17 out of 28 specimens of intact mucosae, leucocytes could be seen migrating between the cornified cells. The changes in the vagina are similar to the keratinization found by Wolbach and Howe (15) for other epithelial tissues of the body.

Mason finds that testicular damage occurs in the absence of vitamin A (12). He has observed considerable testicular damage, especially in chronic deficiency of vitamin A of a mild type, without xerophthalmia (personal communication). In the female rat, similarly, vaginal changes precede other symptoms of avitaminosis of A.

There is a definite sequence with which various structures of the body are affected by an inadequate amount of vitamin A. It may be that the vagina becomes affected first because it represents unusually active epithelium. Or again, the cells of all of the affected membranes may become keratinized at about the same time. Cornification may appear earlier in those membranes most exposed to drying, as the vagina and cornea, but xerophthalmia may not be apparent until the cornified cells of the eyelids have become the seat of infection.

Conclusion

Continual cornified vaginal cells appeared in 100 per cent of the rats on diets deficient in vitamin A, where the animals lived to show avitaminosis. Histological section of the vaginas of 33 rats showed the peripheral cells of the vagina to be cornified. Cornification of the vagina invariably preceded other symptoms of avitaminosis; the time elapsing was positively correlated with the amount of vitamin contained in the diet or stored in the animal. In this group of 60 rats cornified cells in the vagina were the most delicate indication of avitaminosis of A.

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STANDARDS FOR PREDICTING BASAL METABOLISM

I. PREDICTION FOR GIRLS FROM 17 TO 21

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THE adequate defining of physiological normals in the field of basal metabolism has been of necessity a slow process because of the multitude of factors which can modify the findings, many of them subtle and difficult to evaluate. Both the greater difficulty of standardizing experimental conditions with immature subjects, and the greater physiological variability of the growing periods have retarded knowledge of the exact configuration of the age-curve of metabolism in childhood and youth. There are several interesting details of this curve which await clearer definition by the compiling of statistically significant volumes of normal data. Among these is the period around the age of puberty. Another is the point where the slope of the curve flattens out as the adult type of metabolism becomes stabilized. It is with the latter part of the curve for women that the present study is concerned.

The study was undertaken in the effort to determine the reference standards most suitable for practical use in connection with student health at the University of Wisconsin. In such an institution young women come in for a large share of the metabolic studies that are carried on. Interpretation of results has been greatly hampered by the serious inconsistencies of available predictions for such subjects. These inconsistencies can be traced both to relative paucity of data for ages between 17 and 21 at the time when the classical reference standards were being formulated, and to wide differences in methods that were resorted to for temporarily bridging the gap.

The three predictions from authoritative sources which are most widely referred to in America have here been considered: the lower ranges of the original Aub-DuBois tables (1); the special prediction for girls from 12 through 20 proposed by Benedict in 1924 (4); and the Harris-Benedict prediction for adult women (15), after extrapolating for ages under 21. In endeavoring to compare these it becomes evident that not only do wide discrepancies in average absolute level exist between their predictions,

but the values of the Benedict prediction, built up by methods of study very different from any other standard in the field, do not run parallel to those of either of the others for girls of different body builds. Thus a girl of 17, 157 cm. tall and weighing 47.3 kg., whose measured heat-production is found to be 1166 calories for 24 hours would be reported as showing a metabolic rate of −16 by DuBois, −12 by Harris-Benedict, and +13 by the Benedict prediction; whereas a girl of 18 years, 157 cm., and 66.4 kg., with heat-production determined experimentally as 1387 calories per 24 hours would show rates within the "normal" range by all three predictions, i.e., −9 by DuBois, −7 by Harris-Benedict, and −4 by Benedict.

Being unable to make a decision between these on purely theoretical grounds, we set out to collect a sufficiently large number of data on normal girls under 21 to make possible either a convincing choice between the older standards, to indicate how one of these could be modified to meet our needs, or, if necessary, to serve as basis for formulating a new standard of reference in clinical cases from the same source as the normals studied. This last was made possible through the coöperation of the Department of Mathematics of the University, after data were on hand for 97 girls of ages quite uniformly distributed between the years 17, 18, 19 and 20. This standard has been included with the three older ones in various comparisons on our own test data and finally on a comparably large series of normal controls in the same age range reported in several recent studies from widely different localities.

The results of these various studies and comparisons have encouraged us to believe that our data are of more than local significance. In offering the prediction standard we do so primarily for its immediate usefulness and for the interest of the comparisons upon which it was based and which it may suggest. The gain in prediction accuracy which it offers over the best of the older standards, after correcting the latter for differences in mean absolute level, is slight, though consistent for both the Wisconsin and other data considered. The comparisons demonstrate the logic of considering girls of college age along with adult women in attempting to predict their metabolism.

It is generally conceded that the standards for adult women are too high (22, 5, 13), and this has been found particularly true in our hands for younger women. But permanent recommendations for correcting the older standards can be justified only after many more data have been compared over the largest possible range of ages. There is no assurance that any single recommendation for correction of mean values of any of the stand-

ards will be valid for all age-ranges or even for different groups of controls of the same ages and differing in average bodily configuration. The present comparative study is offered for whatever bearing it may have in the eventual reaching of some general agreement on the subject of standards for women.

Experimental

The rather large bulk of fundamental data connected with the study has been put on record in the *Transactions of the Wisconsin Academy for Sciences, Arts and Letters* for 1932 for the interest of those who may care to make more detailed examination of the individual measurements or to use any parts of the data for comparisons.

The subjects were students who entered the University with a grading of A in their medical and physical examinations and whose histories indicated good health since coming to school. Cervical inspection and supplementary questioning of each subject at the time of her test were designed particularly to rule out suspicion of thyroid disease or history of significant menstrual irregularity or difficulty other than common degrees of discomfort. Experience showed it to be undesirable to attempt to get satisfactory results at certain times of the school year, such as periods soon before or after vacations or examinations, prominent social events, or athletic exhibitions in which subjects who came from the Physical Education Department were participating. This conclusion was reached after it became apparent that rates obtained at such times were apt to be distinctly high in comparison with later data on the same subjects, while pulse-rates and erratic respiration often pointed to inadequate relaxation.

Our aim was for essentially uniform distribution of ages. In the data submitted to the final analysis there were represented 25 subjects each of the ages 17, 18 and 20; and 22 of age 19, including in each classification all those within 6 months of the designated year. A total of 163 acceptable tests was obtained on the 97 subjects. Intervals between tests were 3 weeks in 35, and 7 to 55 days in the remainder. First accepted tests only on each subject were used for the prediction standard, for reasons that will be mentioned later. The other tests were used for making various comparisons.

Geographic source of subjects.—Eighty per cent of the students were drafted from newly entering classes, so that their prevailing living conditions can with fair safety be considered as determined by their homes. The mid-west of course predominated largely, furnishing 85 per cent of the

subjects, but 17 states in all were represented. Roughly ten times as many of the subjects were from goiter-belt as from non-goiter-belt regions (23), so that no formal comparisons were justified. It was of passing interest, however, to find that plus and minus metabolic rates (Wisconsin standard) were similarly distributed in the two groups.

College classification. One-third of the subjects were registered in the Physical Education course at the University. This relative preponderance may be attributed to several factors: the physical fitness of these girls, their early registration, and their willingness to cooperate. When the metabolic rates of these subjects were tabulated separately from those of the rest of the group, no distinctive tendencies to high, low, or unusually variable rates could be detected. Any actual tendency to greater variability in heat-production, sometimes said to obtain for the physically active, in distinction to relatively more sedentary types of individuals, could perhaps be offset in this particular group as a whole by the stabilizing effect of more perfect relaxation as the result both of physical discipline and ready understanding of the aims of such an experiment.

Thyroid observations. The criteria of what constitutes a strictly "normal" thyroid gland must vary somewhat according to the region in which the observations are made. Someone has observed that some degree of enlargement of the gland is so common in young women in our middle states that even those illustrated on magazine covers are apt to show slight evidence of goiter! Certainly it would have been impossible for us to assemble any significant sized group for study if we had discriminated against simple enlargement of the gland. Hence, as long as all our other criteria of normal health were satisfied—which excluded carefully, of course, all suspicion of *symptoms* referable to the thyroid—we accepted the girls as they came to us. When the results of the special examinations made at the time of the tests were tabulated it was found that only 2 of the 97 girls were recorded as having strictly negative thyroids—i.e., neither isthmus nor lobes found visible or palpable. On the other hand, there were 24 (26 per cent) in whom enlargement was definite enough to be considered in the author's judgment as mild or moderate degrees of simple goiter. All of the latter were from regions classed as goiter-belt or border-line. They did not show any definite trend in metabolic rates, as a group.

Most of the subjects from goiter-belt regions have histories of having used iodine in some form as goiter prophylaxis, usually for short periods only. If such medication serves its admitted purpose—i.e., of replacing an element deficient in the natural ration, it should presumably tend to make

this group if anything more comparable, than otherwise, with those from regions where the necessary iodine is obtained in the food.

Menstruation. After consultation with members of the clinical staff, it was decided to eliminate results on any subjects who gave history of irregularity in periods of a week or more, or of dysmenorrhea sufficient to be admitted as incapacitating for any time. The data on subjects whose tests were satisfactory but who had to be discarded as not strictly normal by these requirements have been set aside for future study, since one of the problems in which we are specifically interested is the connection between basal metabolic rate and menstrual history in women not definitely in ill health.

Catamenia without discomfort at the time of a test we did not allow to disqualify the test. The state of the literature on this subject at the time our investigation was undertaken did not suggest that this discrimination would be necessary or even desirable in a study of this sort. As the study progressed, the thoroughgoing investigations published by Benedict and Finn in 1928 (6), by Hitchcock and Wardwell (16) and by Griffith *et al.* in 1929 (14), and by Conklin and McClendon in 1930 (9), served to crystallize the general impression derived from earlier reported work, namely that cyclic changes in the heat-production of women can be demonstrated by adequate methods of study and analysis. The very difficulties that have beset the final demonstration of this periodicity point to the fact that the cyclic changes are of a relatively minor order of magnitude, and that the maxima and minima vary greatly in their position in the cycle from one woman to another, and even in different cycles in the same woman. Apparently the changes in metabolic rate that can be correlated with the menstrual cycle consist of a low phase either during or shortly following the period, with a high one of about the same order of magnitude recurring some time intermenstrually. The position of the intermenstrual rise has been variously placed as about mid-way between the periods, or shortly before the beginning of a period—i.e., pre-menstrually. Since there are both maxima and minima of about the same relative extent, it would hardly seem more logical to try to avoid the menstrual (or post-menstrual) low points than the intermenstrual high ones in a study that aims to define reasonable ranges of variation of the basal metabolism of normal women for purposes of prediction. If one avoided both types of changes, there would be left only a short intermenstrual interval for making the tests, and there would still be decided uncertainty as to just which interval this would be.

It seems desirable, however, to record the phase of the menstrual cycle at which tests are made in women. This we have done in the majority of the present tests. Perhaps future studies will confirm the suspicion voiced by Lanz in 1925 (21) that though such cyclic changes are apparently of minor importance in normal women, they may perhaps become exaggerated not infrequently in those who are ill, particularly with some of the endocrine disorders.

Technic. The tests were made in the metabolism laboratory of the Wisconsin General Hospital under the same general conditions used for the study of out-patients, but in the interest of unity and control in the series, were handled with very rare exceptions by the same operator. Appointments were made between 7:30 and 8:30 a.m. Each student who was given an appointment, was furnished with the instructions for preparation and brief description of the routine which the hospital provides for patients who come in for the test. The usual requirements for preparation were followed—i.e., the subjects came without breakfast, and were required to lie quietly with enough covering to be comfortable, for at least 30 minutes before the test.

A closed circuit type of apparatus was used—in an occasional test at the beginning of the series the "Sanborn Grafic" was used, but for all the rest, the Benedict "Portable" model with motor-driven circulation. The machines were kept at all times free from leaks or other determinable defects. During each run a confirmatory leak-test was made by Benedict's expedient of placing a weight on the spirometer midway through the run and noting the point in the tracing where the weight was applied.

At least two technically acceptable runs were required for each test. The lowest run was accepted as determining the rate for any given test, but all accepted runs were figured separately for the information to be gained by their comparison. This is our routine practice, since with the technic used we feel that any error which might conceivably arise without being detected would tend to raise, rather than lower the readings.

All tests were accepted for analysis unless specific grounds for their rejection were apparent in technical flaw, lack of coöperation or indisposition of the subject at the time of the test. One of the things we were interested in testing out was whether ranges of variation similar to those considered reasonable in work with adults would be found in these measurements of younger subjects.

Measurements taken were defined as follows: the body *weights* recorded were nude weights. *Age* was taken to the nearest birthday. In view of the

emphasis that has been placed by many observers upon the value of the *sitting height* for making physiological comparisons, this measurement was taken for these subjects in addition to the *standing height*, in case the former should prove to furnish the more accurate factor for prediction of the metabolism. *Calories* of "basal" heat-production were calculated from the oxygen consumption as measured during the lowest run within any given test, by the usual assumption of the average post-absorptive respiratory quotient for which the caloric equivalent of oxygen is 4.825 per liter (8). In calculating calories per square meter, *surface area* has been estimated by the height-weight chart of DuBois and DuBois (11). *Metabolic rates* have been figured in each test according to 4 predictions including our own, which will be discussed later.

Results

I. General Comparisons Among Wisconsin Test Data

The agreement between duplicate runs within tests was figured in 170 tests on the 97 subjects of the standard series, plus 7 examined after the data for the standard were under analysis. Percentage differences were figured on the basis of the lowest run in each case. Twenty-six tests showed no difference between successive runs. Of the rest, 82 showed lowest readings in the first runs, and 62 in runs after the first. In 72 per cent of the total number the discrepancies were within 3 per cent *of the low run;* in 88 per cent within 5 per cent; and in all, within 10 per cent. Our impression gained from general experience with untrained adult subjects is here confirmed—namely, that duplicate runs in the large majority of well-controlled tests can be expected to agree within 5 per cent of the lower run, while there will always be some larger differences that are not definitely accounted for.

Agreement between repeated tests on the same individuals. Of the 70 instances in which more than one acceptable test per subject was obtained, 30 showed lower figures for oxygen consumption in the first tests, and 40 in later ones. The magnitude of the discrepancies was in this case calculated on the basis of the first accepted test for the individual—that is, the figures show changes in measured heat production after the first tests. In 47 per cent of the cases these changes were within 3 per cent of the first results; in 64 per cent within 5 per cent; and in 93 per cent within 10 per cent.

Pulse-rates were counted routinely twice during each run for supplementary evidence as to the success with which relaxation was maintained. The following average rates observed in the different age-groups of the Wisconsin series suggest that on the whole relaxation was maintained

almost as well in first as in later tests. In individual cases of course there was much variation in both directions from the picture for the group, since the pulse-rate and metabolic rate, as is well known, vary only roughly parallel with each other.

TABLE I

AVERAGE PULSE-RATES—WISCONSIN STD. SERIES.

Age	All first tests:		Subjects accepted more than once	
	Aver.	Range	Aver. first tests	Aver. later tests
17	69	48–86	70	68
18	69	53–86	69	69
19	68	43–89	71	63
20	72	59–88	71	69
All:	70	43–89	70	68

It is interesting to note that the minimum which we found in three of these four groups of healthy American girls is lower than the minimum (54 p.m.) found by MacLeod, Crofts, and Benedict (22) in their study of 9 Oriental young women who had lived in this country for from 15 months to 4.5 years. The average tendency for what we consider low levels of such vital functions as pulse and metabolism in Orientals, even after prolonged exposure to our conditions of living, is probably considered most logically as an indication of a constitutionally superior capacity for repose on the part of these races, rather than an essential difference in metabolic processes. It would appear, then, that a good many of these girls were quite comparable to the Orientals in this respect.

Body build.—Figure 1 gives a picture of the height-weight relationships in the 97 Wisconsin standard subjects. The heavy line shows the average for the group, given by the regression line of weight on height, and the broken lines mark off distances on either side equal to the standard deviation for weight. We were interested to find that the regression equation predicted almost the same weights for height as Bardeen's tables (2), which we use for clinical standards. The Wisconsin group is evidently anthropometrically representative.

Menstruation and Metabolic Rates. Of the 97 first tests used for the Wisconsin Standard, 69 have been placed as to time since the beginning of the last menstrual period. Probably few of the unplaced ones were on menstrual days, since the girls who came during the catamenia usually volun-

teered that information. Considering all tests on the standard and extra subjects examined by the author, information is at hand for identifying time in the cycle of 114 tests. The cycle was divided into 5-day periods, including the first 5 as menstrual days, and average metabolic rates according to the Wisconsin standard were computed for each interval.

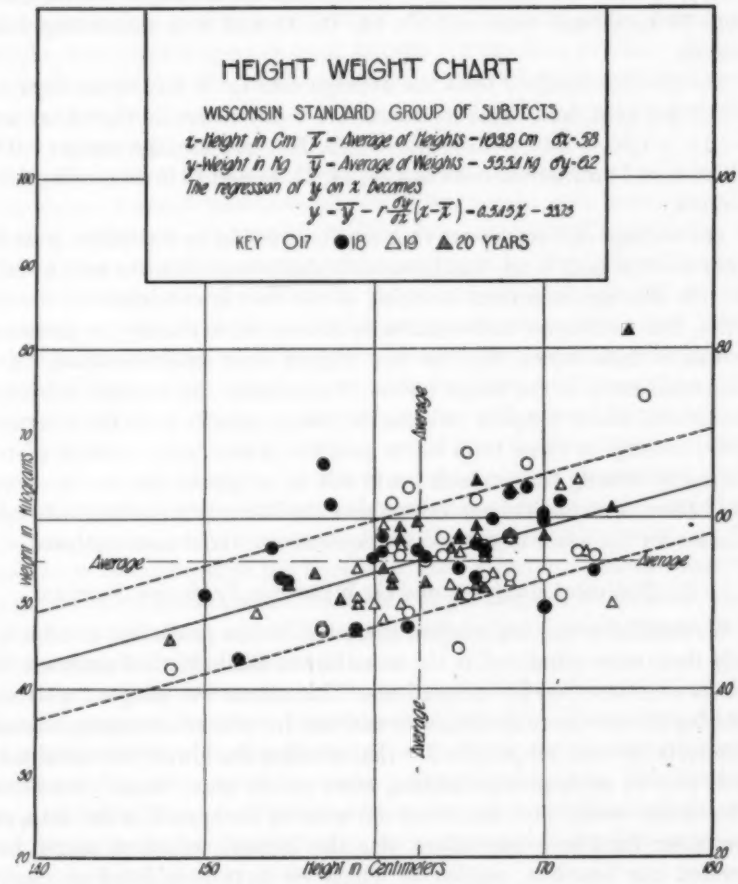

FIG. 1

Though little information was anticipated from the averaging of such scattered tests, there nevertheless was indicated a tendency to values somewhat lower than the intermenstrual average during or immediately follow-

ing the period. The exact time of an intermenstrual high point, also indicated here, varies according to the grouping of days chosen for averaging. The following values were obtained:

For the 69 first tests: the average rate for 5 menstrual days was −2.20 per cent, and those for successive 5-day intervals thereafter were −2.70, +2.07, +.075, +1.18, and ±0. Intermenstrual average: +0.12. There were 10 menstrual tests and 10, 14, 16, 11 and 8 in succeeding 5-day periods.

For all 114 classified tests the average rate for 5 menstrual days was −2.21 per cent, and those for successive 5-day intervals thereafter were −2.13, +1.96, +1.48, −1.06, and −1.15. Intermenstrual average: −0.07. There were 14 menstrual tests and 24, 24, 21, 18 and 13 in succeeding 5-day periods.

The average differences are thus small, as would be inevitable from the large effect of both inter- and intra-individual variabilities in such a series, but the average menstrual lowering of the rate is consistent in the two series. The two lowest individual rates did not occur during the menstrual period in these series, but the two highest were intermenstrual. Of 14 menstrual rates in the larger series, 10 are below the average Wisconsin prediction, which happens to coincide almost exactly with the intermenstrual average of those tests whose position in the cycle could be placed. Evidently among those which could not be so placed by the records at hand there were just enough rates above the Wisconsin prediction to compensate for the lower menstrual averages among those tests analysed.

II. Statistical Analyses, and the Wisconsin Prediction Standard

We decided to use *first accepted tests only*, for the prediction standard, so only these were submitted to the more formal mathematical analyses. The others were reserved for comparisons. This course was adopted after considering the two more obvious alternatives: 1.—that of averaging all available tests for each subject; or 2.—that of using the lowest test obtained in each case as perhaps representing more nearly true "basal" conditions. The former would have smoothed out some of the spread in the data, and the latter (and to a less extent also the former) would of course have lowered our base-line, neither of which we have considered necessarily desirable from the point of view of prediction. Either, to be strictly applied, would have required our having more than one acceptable test on every individual in the series, which, however, we had not set as our goal. The idea that underlay our decision was that we were interested primarily in

arriving at some concrete ideas, not of ideal rates of heat-production, and not only of their average probable level, but rather in the whole *range* of variability within which individual determinations might reasonably be expected to fall for subjects of these ages not especially trained, but studied under well-controlled standard "basal" conditions.

After some rather extensive preliminary analyses, the method of multiple correlation, making metabolism a linear function of the height and weight, was decided upon as most suitable for the data at hand.[1] Average measurements and the most important of the statistical constants calculated for the 97 sets of measurements considered by separate and combined age-groups may be found elsewhere (25).

For the first two groups that were completed for analysis, equations were formulated in which measurements for sitting height were incorporated as well as, or instead of, standing height measurements. Comparisons, including those of the standard errors of estimate by the different types of equation, made it evident that the use of the sitting height did not add anything of value from the point of view of accuracy in predicting. Therefore the less usual and probably less accurate measurement was dropped.

While 25 cases for each year proved to be ample for defining the influence of height and weight on the metabolism with a good degree of practical certainty, a much larger number would have been necessary to determine an age-factor within the narrow range of ages of the present study. The changes in heat-production with each year of age over this period are evidently so small as to be readily obscured by incidental fluctuations. We shall hope to incorporate the benefit of such a factor when our observations shall have been extended to cover a larger range of years. Meanwhile, for practical purposes of prediction, the age-factor is small enough to be safely neglected within this range.

In a general way these data show an unmistakable downward trend of heat-production from the younger to the older girls. This is brought out most consistently when the 17 and 18, and the 19 and 20 year groups are combined to smooth out some of the individual variations. This has been done in Table II for the data of all tests of our series. For logical inter-individual comparison, heat-production is expressed in the final column in calories per unit of body surface.

One of the less obvious pitfalls in trying to define small differences in

[1] Curves obtained by expressing the logarithm of the metabolism as a linear function of the logarithms of the height and weight proved to be sensibly the same as those based on the simple measurements for the ranges of values dealt with.

measured heat-production from one group to another is suggested when we examine the averages of first and later tests separately. Inspection of Table II reveals a considerably larger drop between first and repeated tests in the younger than the older girls. It appears probable that some part of the observed difference in average levels of heat-production, therefore, is apparent, and due to better relaxation on the part of the older girls as a group.

TABLE II

AVERAGE HEAT-PRODUCTION OF GIRLS 17–18 AND 19–20 YEARS OLD
All tests Wisconsin Standard Series

Subjects		Ages	No. of subjects in group	No. of tests in group	Average calories/ 24 hrs.	Average cal./Sq. M./ hour
All Tests, Subjects who contrib. to U. W. Std.		17–18	50	89	1313	34.40
		19–20	47	74	1280	33.54
Those who had more than one test	First tests	17–18	39	39	1330	35.00
		19–20	25	25	1284	33.57
	Later tests	17–18	39	39	1304	34.14
		19–20	25	27	1272	33.43

Our final decision was to incorporate the combined data for the 4 years from 17 through 20 into the simple prediction standard given as Table III. The prediction is based upon standard methods of multiple correlation and represents the equation:

Basal heat production in calories for 24 hours = 10.63 × weight in kg. + 3.23 × height in cm. + 184.61.

III. Comparisons of Suitability of Various Standards for Predicting the Heat Production of Girls from 17–21.

A composite set of data on 119 normal controls has been collected for concrete comparisons as to the suitability of different prediction standards for girls of the ages we have studied. In order to come to any decision as to the suitability of our own prediction, which we preferred to use until there could be some more general agreement on the manner and extent to which existing standards must be modified to meet more modern conditions, of course it was necessary to try it on at least a comparably large number of data other than those from which it was constructed. We began by assembling what records we could on other normal controls of the same ages

TABLE III

PREDICTION TABLE FOR BASAL METABOLISM OF YOUNG WOMEN—AGES 17–21
University of Wisconsin, 1930
Height in Centimeters

Weight in Kilograms	140	145	150	155	160	165	170	175	180
40	1062	1078	1094	1110	1127	1143	1159	1175	1191
41	1073	1089	1105	1121	1137	1153	1170	1186	1202
42	1083	1099	1116	1132	1148	1164	1180	1196	1212
43	1094	1110	1126	1142	1158	1175	1191	1207	1223
44	1105	1121	1137	1153	1169	1185	1201	1218	1234
45	1115	1131	1147	1164	1180	1196	1212	1228	1244
46	1126	1142	1158	1174	1190	1207	1223	1239	1255
47	1136	1153	1169	1185	1201	1217	1233	1249	1266
48	1147	1163	1179	1196	1212	1228	1244	1260	1276
49	1158	1174	1190	1206	1222	1238	1255	1271	1287
50	1168	1184	1201	1217	1233	1249	1265	1281	1298
51	1179	1195	1211	1227	1244	1260	1276	1292	1308
52	1190	1206	1222	1238	1254	1270	1286	1303	1319
53	1200	1216	1232	1249	1265	1281	1297	1313	1329
54	1211	1227	1243	1259	1275	1292	1308	1324	1340
55	1221	1238	1254	1270	1286	1302	1318	1335	1351
56	1232	1248	1264	1281	1297	1313	1329	1345	1361
57	1243	1259	1275	1291	1307	1323	1340	1356	1372
58	1253	1270	1286	1302	1318	1334	1350	1366	1383
59	1264	1280	1296	1312	1329	1345	1361	1377	1393
60	1275	1291	1307	1323	1339	1355	1372	1388	1404
61	1285	1301	1318	1334	1350	1366	1382	1398	1414
62	1296	1312	1328	1344	1360	1377	1393	1409	1425
63	1306	1323	1339	1355	1371	1387	1403	1420	1436
64	1317	1333	1349	1366	1382	1398	1414	1430	1446
65	1328	1344	1360	1376	1392	1409	1425	1441	1457
66	1338	1355	1371	1387	1403	1419	1435	1451	1468
67	1349	1365	1381	1397	1414	1430	1446	1462	1478
68	1360	1376	1392	1408	1424	1440	1457	1473	1489
69	1370	1386	1403	1419	1435	1451	1567	1483	1499
70	1381	1397	1413	1429	1446	1462	1478	1494	1510
71	1392	1408	1424	1440	1456	1472	1488	1505	1521
72	1402	1418	1434	1451	1467	1483	1499	1515	1531
73	1413	1429	1445	1461	1477	1494	1510	1526	1542
74	1423	1440	1456	1472	1488	1504	1520	1536	1553
75	1434	1450	1466	1483	1499	1515	1531	1547	1563
76	1445	1461	1477	1493	1509	1525	1542	1558	1574
77	1455	1471	1488	1504	1520	1536	1552	1568	1585
78	1466	1482	1498	1514	1531	1547	1562	1579	1595
79	1477	1493	1509	1525	1541	1557	1573	1590	1606
80	1487	1503	1520	1536	1552	1568	1584	1600	1616

at this university. Besides the 7 subjects studied after the data for the standard were under analysis, there chanced to be available measurements made in connection with two graduate theses. These dealt with a group of 21 presumably normal sorority girls studied by Jean Fish in 1928 for a thesis in psychology; and a series of 9 presumably normal students investigated by W. C. Shipley in 1930, also in connection with a problem in psychology. These together constitute a supplementary Wisconsin series of 37 cases. The two psychology studies were made independent of the hospital under carefully controlled experimental conditions, for the physiological comparisons which they might afford.

For a more crucial test we have used two large series of controls which came to our attention in 1930 and 1931 in reports of the metabolism of young women from non-goiter-belt regions. These are the study of the basal metabolism of young college women in Florida by Jennie Tilt (26), and a study by Remington and Culp (24) of the basal metabolic rates of student nurses at Charleston, South Carolina. Individual measurements, rather than merely group averages, were fortunately available in these reports.

Twenty-nine of Tilt's subjects were between 17 and 20.5 years, and hence comparable with our group. The technic used was similar to ours and Miss Tilt calculated the rates of her subjects by both the Harris-Benedict and the Aub-DuBois predictions. By both standards the rates of most of the subjects were decidedly low, and the author concluded that the basal metabolism of young women in Florida tends to be significantly lower than that predicted for young women of the same ages and living in the north.

Of the 93 student nurses examined by Remington and Culp, 48 were within our required age-range, and thus afforded another large homogeneous group for comparison. The technic again was similar in principle to ours. These authors also noted the prevalence of low rates according to the DuBois standard, but having observed that both lower and higher figures have been reported from studies made in northern states, concluded that the low values could not be regional. They suggest nutritional level as a possible factor in trend of metabolic rates, though they feel that this factor must be slight in groups of normal individuals. They did not consider the possible bearing which the choice of standards might have on warranted interpretations.

We were interested to see how our data compared with the more modern studies of normals from the Nutrition Laboratory of Boston, but found just 5 subjects within the range of our study in Benedict's 1928 series of

controls (5). Benedict made a definite effort in this later series to secure a wider range of body builds in his subjects than had been the case in the original Nutrition Laboratory series, since this should make possible better definition of the relationships between metabolism and body measurements when the latter are far from average. Three of these five girls are as it chances, heavy in proportion to their heights. As a separate group the small series is therefore unbalanced; but for this very reason, as well as its source, it has proved to offer some most interesting comparisons.

The fundamental data on the 119 subjects of the above composite series, together with notations of the recalculations and derived data which we have added for comparison are on record in the Transactions of the Wisconsin Academy (25).

Prediction standards compared. Metabolic rates have been calculated as percentage deviations of observed calories from each of 4 predictions, including our own, as noted. The distributions of the individual deviations and the average fit of the 4 predictions are shown graphically in Figures 2, 3 and 4, and the average measurements and calculated rates in all groups of controls given in Table IV. It was not possible to separate the total comparison series into first and later tests since some of the reports gave data already averaged for the individuals. The Benedict prediction (in which metabolism is considered as a simple function of weight, disregarding height, and age in the range from 17 to 21) shows itself decidedly inferior to the other three by excessive scatter of individual rates about the mean. In both series of controls, according to this last prediction, rates run as low as -11 or -12, and as high as $+33$ and $+35$, whereas a much more reasonable total spread is indicated by each of the other three standards.

The general similarity of the distributions according to three of the four predictions suggests immediately the logic of making their zero-points coincide before any really fundamental comparisons are attempted. This we have done by applying the constant percentage corrections needed to center each of the older predictions for our standard data. On this more legitimate basis the rates have been recalculated for those individuals of both series of controls for whom data on first tests are separately available, since averaged measurements would not be strictly comparable with those of our standard series. This leaves a comparison series of 85 individuals. The standards have then been recompared as to: 1.—new algebraic average of individual rates; 2.—standard errors of estimating rates by the various predictions; 3.—percentiles falling within ± 10 per cent and within

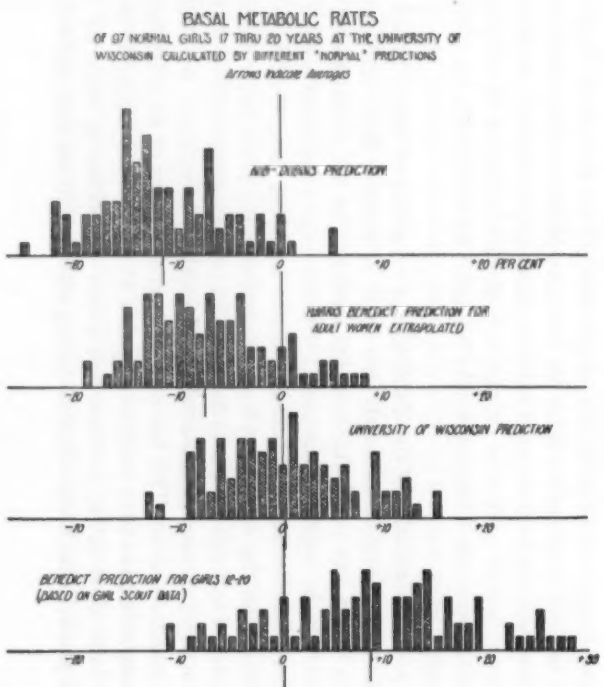
BASAL METABOLIC RATES
OF 97 NORMAL GIRLS 17 THRU 20 YEARS AT THE UNIVERSITY OF WISCONSIN CALCULATED BY DIFFERENT "NORMAL" PREDICTIONS
Arrows indicate Averages
AUB-DUBOIS PREDICTION
-20 -10 0 +10 +20 PER CENT
HARRIS BENEDICT PREDICTION FOR ADULT WOMEN EXTRAPOLATED
-20 -10 0 +10 +20
UNIVERSITY OF WISCONSIN PREDICTION
-20 -10 0 +10 +20
BENEDICT PREDICTION FOR GIRLS 12-20 (BASED ON GIRL SCOUT DATA)
-30 -10 0 +10 +20 +30

FIG. 2

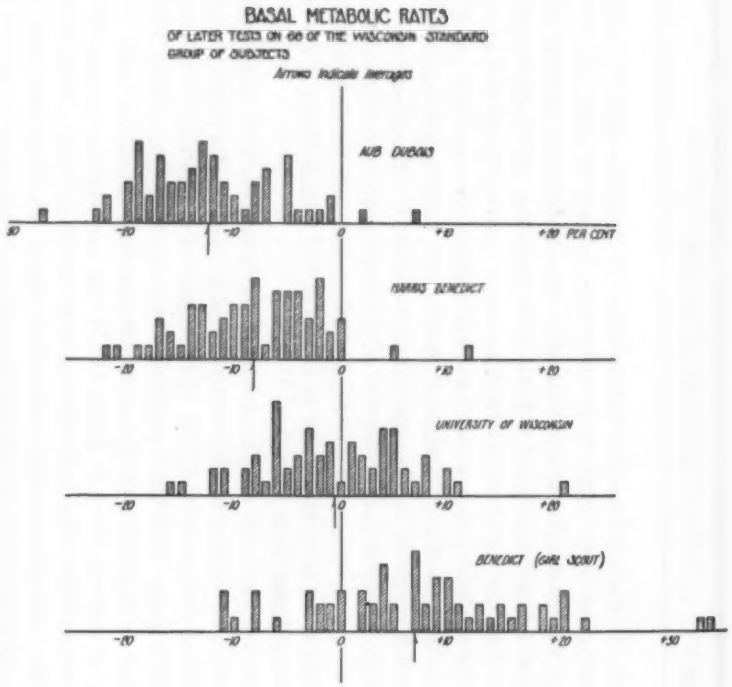
BASAL METABOLIC RATES
OF LATER TESTS ON 66 OF THE WISCONSIN STANDARD GROUP OF SUBJECTS
Arrows indicate Averages
AUB DUBOIS
-30 -20 -10 0 +10 +20 PER CENT
HARRIS BENEDICT
-20 -10 0 +10 +20
UNIVERSITY OF WISCONSIN
-20 -10 0 +10 +20
BENEDICT (GIRL SCOUT)
-20 -10 0 +10 +20 +30

FIG. 3

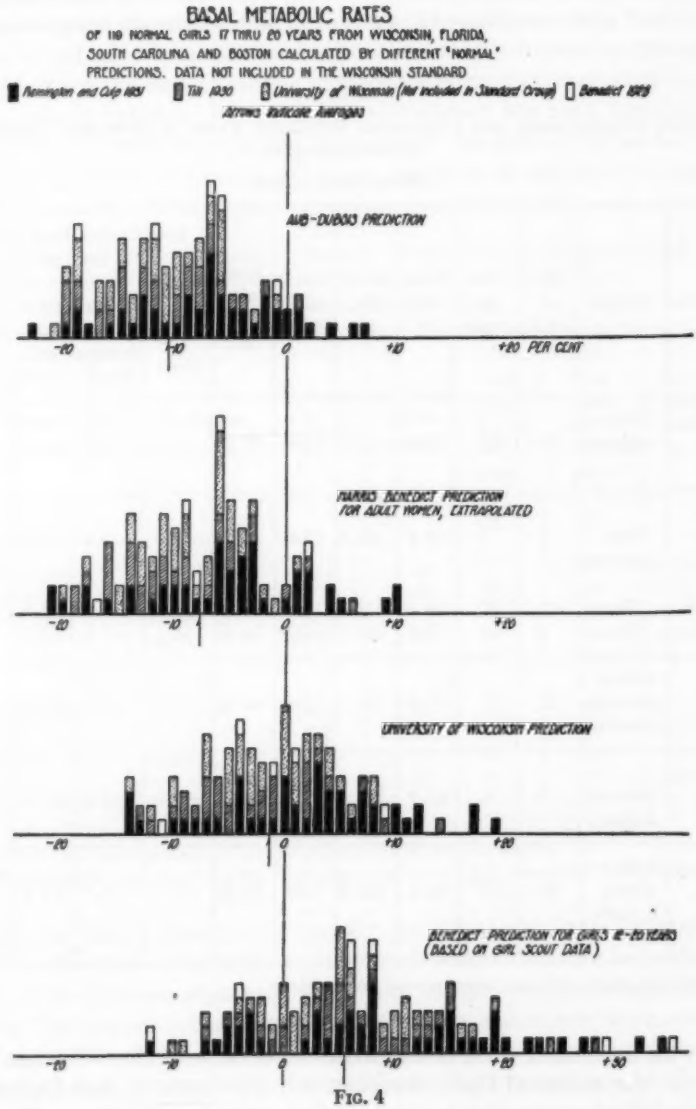
BASAL METABOLIC RATES
OF 110 NORMAL GIRLS 17 THRU 20 YEARS FROM WISCONSIN, FLORIDA, SOUTH CAROLINA AND BOSTON CALCULATED BY DIFFERENT "NORMAL" PREDICTIONS. DATA NOT INCLUDED IN THE WISCONSIN STANDARD
Rennington and Cole 1931
Tilt 1930
University of Wisconsin (Not included in Standard Group)
Benedict 1919
Arrows Indicate Averages
AUB-DUBOIS PREDICTION
-20 -10 0 +10 +20 PER CENT
HARRIS BENEDICT PREDICTION FOR ADULT WOMEN, EXTRAPOLATED
-20 -10 0 +10 +20
UNIVERSITY OF WISCONSIN PREDICTION
-20 -10 0 +10 +20
BENEDICT PREDICTION FOR GIRLS 16-19 YEARS (BASED ON GIRL SCOUT DATA)
-20 -10 0 +10 +20 +30

FIG. 4

±15 per cent of the respective predictions; and 4.—extreme ranges of individual rates outside ±10 per cent. The findings are summarized in Table V.

TABLE IV

AVERAGE MEASUREMENTS AND CALCULATED METABOLIC RATES IN DIFFERENT GROUPS OF NORMAL CONTROLS

(All tests each subject)

Source	Subjects	No. of subj.	No. of tests	Aver. Wt., kg.	Aver. Ht., cm.	Aver. cals./ 24 hrs.	Aver. cals./ Sq.M./ hr.	Aver. Metab. Rate acc. to Pred. of:			
								Aub-Du-Bois.	Harris-Ben., extrapolated.	Benedict, girls 12–20	Univ. Wis., girls 17–20
U. W. std. subj.	Univ. students	97	163	55.63	163.5	1296	34.01	−11.9	−7.6	+7.7	−0.3
U. W. suppl.: M.E.S.	Univ. students	7	7	56.9	165.8	1300	33.31	−15.0	−8.4	+5.0	−2.0
J. Fish	Do.	21	21	54.8	162.4	1266	33.61	−11.0	−9.0	+7.0	−1.8
Shipley	Do.	9	13	57.5	162.7	1297	33.54	−10.1	−7.8	+3.9	−0.2
Tilt, 1930	College students, Florida	29	*	53.4	159.4	1235	33.40	−11.6	−10.0	+6.5	−2.6
Benedict, 1928	Normals, Boston.	5	*	60.9	161.0	1336	34.04	−9.0	−7.6	+0.8	−1.2
Rem. & Culp, 1931	Stud. nurses, S. Carolina	48	138	56.1	162.9	1320	34.56	−8.1	−6.1	+8.5	−1.0

* Each test reported = average of several observations.

By all the criteria there is very little choice between the Wisconsin prediction, the corrected Harris-Benedict and the corrected Aub-DuBois in success of predicting for both groups of controls, though in all-round accuracy of fit they arrange themselves consistently in the order named.

By each of the criteria the excessive spread of rates as figured by the Benedict prediction for girls is again brought out.

As a final test of the various standards, we were interested to find which would afford the best fit for the individuals of atypical body-build in the two series of controls. Superiority in this respect has been variously ascribed to the DuBois prediction (presumably because of the surface area concept included, and because it is not statistical in nature (19)), and to

TABLE V

COMPARISONS OF "FIT" OF DIFFERENT "NORMAL" PREDICTIONS OF METABOLIC RATES WHEN OLDER STANDARDS ARE CENTERED TO FIT AVERAGE U. W. DATA

(First tests only each individual)

Source	No.	Prediction	Algebraic aver. of rates	Std. Error of estim. rates	Of total rates within ±10% of pred.	Of total rates within ±15% of pred.	Extreme rates, (outside ±10%)
					%	%	
U. W. std. subjects—(tests used for std.)	97	Univ. Wis., Girls 17–20	(+0.1)	6.54	87	100	+15, −13
		H–B, Corr.*	(−0.2)	6.57	88	99	+16, −13
		DuBois, Corr.**	(+0.5)	7.13	84	98	+19, −15
		Ben., Corr.***	(+0.4)	8.67	74	89	+19, −18
Other controls: (U. W. Suppl: 37; Rem. & Culp: 48.)	85	Univ. Wis. Girls 17–20	+0.1	7.07	86	96	+19, −14
		H–B, Corr.*	+0.4	7.26	86	96	+18, −15
		DuBois, Corr.**	+3.1	7.96	84	95	+22, −13
		Ben., Corr.***	+0.02	8.90	75	91	+25, −19

* Harris-Benedict extrapolated, corrected by −7% to center for U. W. Std. series.

** Aub-DuBois corrected by −12% to center for U. W. Standard series.

*** Benedict's prediction for girls, 12–20, corrected by +8% to center for U. W. Std. series.

either the Harris-Benedict or the Dreyer standards (because they *are* statistical in nature!) It is obvious that such comparisons can be legitimate only if the different standards start from approximately the same mean levels. For this comparison we picked out those individuals from our two test series who were judged to differ significantly in proportion of weight for height from the majority of their fellows. For convenience we did this by taking those points which fell outside (or just at the border of) the standard area delineated on Figure 1, and by fitting the data of the com-

parison series to the chart in the same way. By this method there were set apart 17 subjects in our group and 21 from the total comparison series. Of these, 25 were overweight for their height by from 15 to 47 pounds according to our standard regression equation, and by from 12 to 40 pounds by Bardeen's tables. The other 13 were underweight by 12 to 34 pounds by our formula, and by 13 to 35 pounds by Bardeen. The groups are not large enough to justify the calculation of statistical constants, but general comparisons involving average and extreme findings are interesting.

The average metabolic "rates" as calculated for both underweight and overweight groups were quite similar to the corresponding figures for the total groups when calculated according to either the original Aub-DuBois or Harris-Benedict predictions. Calories per square meter per hour averaged 34.49 for the two overweight groups, and 34.68 for the underweight, against a grand average for all controls of 34.11. Thus the burden of evidence suggests that these light- and heavy-weight sub-groups are not essentially abnormal in rates of heat-production when compared with the populations from which they were drawn.

The Benedict prediction for girls is at odds with these comparisons in showing distinctly different types of rates for the two types of body build. Thus for the over-weight girls the calculated rates turned out to be close to the "normal" expectation by this prediction, averaging -1.9 in the Wisconsin group, and -2.1 in the other group, with a total range of only $+11$ to -14. On the other hand, the underweight girls consistently showed high rates, averaging $+18$ in the Wisconsin group, and $+26.8$ in the other group, with total range from $+7$ to $+35$. The excellent average fit afforded by Benedict's standard for his own small group of predominantly overweight girls, alone among the groups included in the present study, is therefore not surprising (Table IV). Though this standard may be valid for girls between 12 and 17, which ages furnished the data it was based on, its extrapolation for subjects from 17 to 21 has by no means appeared justified. Its use in the clinic, either alone or in conjunction with other standards, has led to much confusion, which the above observations on normal girls help to explain.

When the older standards had been centered for the Wisconsin data, the results shown in Table VI were obtained.

Again there appears to be little choice between the Wisconsin, the corrected Harris-Benedict, or the corrected Aub-DuBois predictions, assuming that these subjects should show on the average essentially "normal" rates. There is a very slight advantage with the Wisconsin prediction in

smaller total range of individual rates if we consider all the groups compared. The Benedict prediction for girls has been improved by centering

TABLE VI

COMPARISONS OF "FIT" OF DIFFERENT "NORMAL" PREDICTIONS OF METABOLIC RATES FOR OVERWEIGHT AND UNDERWEIGHT INDIVIDUALS FROM VARIOUS SERIES OF NORMAL CONTROLS WHEN OLDER STANDARDS ARE CENTERED TO FIT AVERAGE U. W. DATA

(All tests each individual)

Source	No.	Prediction	Overweight		Underweight	
			Algebraic aver. of rates	Range of rates	Algebraic aver. of rates	Range of rates
U. W. std. subjects	Overweight: 10 Underweight: 7	Univ. Wis., Girls 17–20	+0.2	+12, −8	+1.5	+7, −4
		H–B., Corr.*	+1.6	+13, −8	+2.2	+10, −3
		DuBois, Corr.*	+0.9	+16, −11	−0.3	+6, −8
		Ben., Corr.*	−10.2	+1, −18	+7.9	+15, −1
Other control series**	Overweight: 15 Underweight: 6	Univ. Wis., Girls 17–20	−1.5	+9, −13	+4.2	+9, −6
		H–B., Corr.*	+0.9	+11, −13	+1.6	+9, −9
		DuBois, Corr.*	+2.9	+13, −9	+7.7	+14, −1
		Ben., Corr.*	−9.3	±0, −20	+17.4	+25, +7

* Corrections given with Table V.

** Overweight subjects: U. W. total supplementary series: 4; Tilt: 2; Remington & Culp: 6; Benedict: 3.

Underweight subjects: U. W. Supplementary series: 2; Remington & Culp: 4.

of course only to the extent of distributing the excessive scatter between over-weight and under-weight subjects. There would seem to be no need for considering this standard any longer in the presence of three others which appear superior to it by every criterion applied.

DISCUSSION

The success of predicting for these subjects by the methods of either of the classical adult standards would seem adequately to justify the classing of girls from 17 to 21 with adult women in the matter of the physiology of heat-production. This, Benedict was unwilling to do until more normal data should be accumulated. Though the original Nutrition Laboratory

series of 103 normals on whom the Harris-Benedict equation for women was based included data on 12 girls from 15 to 21, Benedict preferred to restrict the use of his tables to ages from 21 up, and thought it safer tentatively to bridge the then inadequately surveyed transitional years by extrapolating upward the curves obtained on his younger Girl Scouts (3, 4). The Harris-Benedict equation for adult women, extrapolated in a straight line through ages certainly down to 16, affords the most accurate of the three older standards for young women which we have tested.

Various modifications of the original Aub-DuBois or the Harris-Benedict predictions by correction of their constants as suggested from different sources (20, 7, 18) have not had to be considered as separate standards for the present study since the application of the more fundamental tests of fitness involves arbitrary readjusting of their mean absolute levels to make them strictly comparable.

The omission of calculations based on the Dreyer predictions (10) is admitted as regrettable since more and more laboratories appear to be using them with satisfaction. Being based upon the same original data, the mean predictions run essentially the same by the Dreyer and the Harris-Benedict standards. But the different types of statistical treatment given the data must affect the relative success of the two in predicting for individuals whose measurements vary much from the group average. According to Benedict, better success has been attributed by some to the Dreyer equations in predicting for individuals of unusual build, though his comparisons for such subjects among the 1928 series of normals from the Nutrition Laboratory (5) do not show this to be the case.

More recently Jenkins, of the University of Chicago, (17) has compared various predictions for adults and children of both sexes against different published bodies of control data and has concluded that the Dreyer formula using observed weight, as expressed in the tables of Stoner, possesses the most advantages and least disadvantages of any on the list. He bases this decision however on general considerations including simplicity and range of applicability, rather than consistent superiority in relative prediction accuracy by the statistical criteria which he himself sets up. By these purely objective criteria his tables show the Harris-Benedict to be decidedly superior for adult males, on whom the majority of available data has been compiled, while the comparative showings are inconsistent for women and the Harris-Benedict is not available for children. There are very few test data included for girls of the ages of the present study. The fact that women are more variable than men in bodily configuration, and

the definite correlation that exists between metabolism and the more typical measurement of height, suggest that further comparisons are necessary on data for women to justify a general decision in favor of the Dreyer prediction, which leaves the factor of height out of consideration.

The present study is in agreement with that of Jenkins in finding the Aub-DuBois prediction less accurate than the Harris-Benedict by the fundamentally important criterion of total spread of individual deviations expressed by the standard errors of estimate corrected to the same mean. Ultimate choice would logically depend upon whether one prefers the maximum possible accuracy in predicting for individuals, or the general biological significance inferred in some form of prediction using the surface area concept, even with the inevitable sacrifice of some degree of accuracy involved in estimating the surface itself. It seems doubtful that any one standard should be expected to meet universal needs.

Summary and Conclusions

The compiling of a statistically significant number of metabolism measurements on girls from 17 through 20 was undertaken at the University of Wisconsin for the purpose of deciding upon a valid standard of normality for such subjects. This small but important sex and age group represents a gap relatively unexplored by the older data upon which prediction standards still in general use were based. Diverse methods used for tentatively bridging this gap gave rise to predictions which are contradictory and confusing in practice.

Basal heat production was determined in 163 tests on 97 girls whose ages were approximately uniformly distributed over the 4 years from 17 through 20. These were University students who were classed as Grade A in their medical and physical examinations and who were judged by further special examination and questioning at the time of their tests to be free from indisposition or defects that should disqualify them from serving as physiological normals.

The results of the tests were subjected to various types of mathematical analysis and the first accepted tests on the 97 subjects were made the basis of a prediction standard expressing the equation: Basal heat production in calories per 24 hours = 10.63 × weight in kg. + 3.23 × stature in cm. + 184.61. The equation was arrived at by standard methods of multiple correlation.

Comparisons by various criteria were made of the "fit" of this standard and that of the Aub-DuBois, the Harris-Benedict prediction for adult women, extrapolated for these ages, and of Benedict's special prediction

for girls from 12 through 20, when each was applied 1.—to the data of the Wisconsin series; and 2.—to a comparably large series of normal controls of similar ages collected from different observers and widely different localities. The latter included data on 77 young women studied in non-goiter-belt regions of the south. The fit of the different standards was similar for the two series of controls.

As they stand the Aub-DuBois prediction averaged from 8 to 15 per cent and the Harris-Benedict from 6 to 10 per cent too high, while the Benedict averaged from 1 to 8.5 per cent too low to describe the measured metabolism of the various sub-groups of controls tested.

Tests of fitness more fundamental than mere success in predicting average absolute level of heat-production for groups—i.e., standard errors of estimating the rates, percentiles falling within ± 10 per cent and ± 15 per cent of the respective predictions, and extreme ranges of calculated rates, were applied after the older standards had been put on a comparable basis with the Wisconsin standard by applying constant percentage corrections to center them for the Wisconsin data. Finally a special set of comparisons was made for the 38 individuals of the two test series who were atypical in proportion of weight to height.

The results of the various comparisons indicated little choice in accuracy of predicting for either group of controls by the Wisconsin prediction, the corrected Harris-Benedict, or the corrected Aub-DuBois, though by all the criteria taken together, they arranged themselves in the order named. The Benedict prediction compared with the other three showed excessive scatter of individual rates about their mean. According to this standard, but not judged by the others, the underweight girls of these series showed very different types of metabolic rates than the overweight girls.

The older standards should be modified only after many groups of data, covering all ages, can be compared to show the ultimate corrections needed to make them fit the largest possible number of cases. It is hoped that the present study can contribute toward an eventual agreement in this respect. Meanwhile, since the Wisconsin prediction has proved to be valid for these considerable numbers of normal girls examined with comparable technic in both the north and south of America, it is offered for its immediate interest in either clinical or physiological comparisons.

Acknowledgments.—The author wishes to acknowledge gratefully the coöperation of the students who served as subjects for the tests, and in addition to express her indebtedness for the following essential collaboration:

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THE PARALLEL DETERMINATION OF THE RESPIRATORY QUOTIENT AND ALVEOLAR AIR OF MAN IN THE POST-ABSORPTIVE CONDITION*

By

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THE respiratory quotient as determined in short periods is often rendered of doubtful value because of the likelihood of abnormality in breathing. The uncertainty is greatest when, as is not uncommon, single, isolated periods are used as a base line for the study of the effect of a superimposed factor. In successive periods there is usually compensation for the possible over-ventilation in one period by an under-ventilation in a following period, so that the average of several periods made in succession is much to be preferred. When a substance is ingested, and there is a marked rise in the respiratory quotient, the measurements of the respiratory quotient and the gaseous exchange together are not sufficient to aid in interpretation of the results, even when carried out with a type of respiration apparatus in which there is every reason to believe that the breathing is perfectly normal. There exists the possibility of the formation of organic acids in the intermediary metabolism of the material which has been ingested, which may alter the carbon dioxide content of the blood. Ideally, one should have frequent determinations of the carbon-dioxide tension of the arterial blood in order to determine whether the changes in the respiratory quotient are the results of metabolic activities alone or are the results of concomitant changes in the carbon-dioxide content of the blood. However, this is very rarely attempted and cannot be easily performed in humans without disturbing seriously the measurements of the respiratory exchange and their interpretation. It has frequently been shown that the carbon-dioxide tension of the arterial blood and the carbon-dioxide tension of the alveolar air run parallel in normal subjects and are nearly identical, so that the simultaneous determination of the alveolar carbon dioxide ought to throw light upon the normality of the breathing and upon the significance of the respiratory quotients as found.

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The use of the alveolar air as a control upon the normality of the respiratory exchange has been employed by Löb (9), Hindmarsh (5), Marsh (11), Schill and Weiss (14) and Heckscher, Faddersböll and Mogensen (3). Ideally, one should make the measurements of the respiratory exchange and of the composition of the alveolar air simultaneously. This has been done by several workers. Lindhard (8) collected by hand the alveolar air at the end of each expiration. Krogh and Lindhard (7) had an electric automatic arrangement for the collection of alveolar air. Löb (9), Henderson and Haggard (4), Wright and Kremer (15), Regelsberger (13), Marschak (10), and Olivier and Bretey (12) have used various automatic methods or methods employing intermittent collection by hand for obtaining samples of the alveolar air during the periods of measurement of the respiratory exchange. Many of these have given results which were generally lower than the samples obtained by the Haldane-Priestley method. After we had begun our studies, Heckscher, Faddersböll, and Mogensen (3) published their method of obtaining a sample of the alveolar air from a Krogh (6) closed-circuit respiration apparatus with a simultaneous graphic record of the respiration. We have measured the respiratory exchange by an open-circuit arrangement with mouthpiece and at the same time samples of the alveolar air have been collected by the Haldane-Priestley method by a special arrangement. The problem at hand was to determine the course of the alveolar air in connection with the ingestion of small quantities of sugars, with respect to their effect upon the gaseous exchange, particularly the respiratory quotient as compared with findings in experiments in which no dose was given. This paper deals with the results obtained in experiments in the post-absorptive condition that were used as controls for the series in a following paper. As the experiments with the sugars extended over several hours, it was necessary to know what would be the course of the respiratory quotient and of the alveolar air during the same periods of time when no sugars were ingested.

The Respiratory Exchange and Alveolar Air in the Post-absorptive Condition

Method of determining the respiratory exchange.—The respiratory exchange was measured by an apparatus which was used in another study and reported elsewhere (Carpenter and Fox, 1).

Method of collecting alveolar air samples. The special feature of this study was the parallel collection of samples of alveolar air without interrupting the measurement of the respiratory exchange. The alveolar air was sampled according to the Haldane-Priestley principle at the end of a

normal expiration by means of the arrangement shown in Figure 1. A quick-seating valve, *A*, was inserted at a point on the inspiratory side closely adjacent to the mouthpiece, *D*, between the inspiratory and expiratory valves, *B* and *C*, of the system for determining the respiratory exchange. On the expiratory side of the mouthpiece, *D*, was soldered a small copper tube, bent downwards. At the lower end of the copper tube

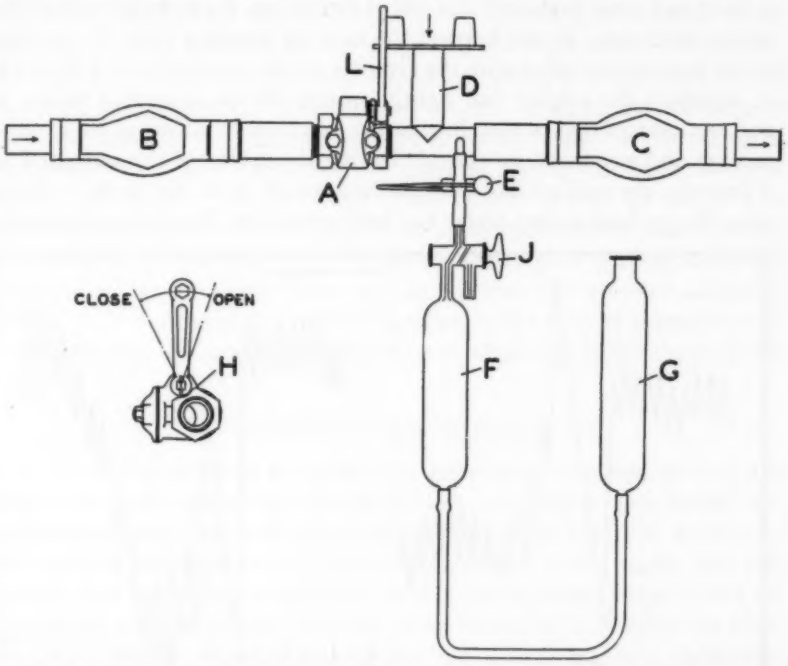

FIG. 1. Arrangement for sampling of alveolar air during the measurement of the respiratory exchange of man. *A*, quick-seating valve between inspiratory and expiratory valves, *B* and *C*. *D*, mouthpiece; *E*, spring pinchcock; *F*, gas sampler with mercury levelling bulb, *G*; *H*, cross section of quick-seating valve; *J*, stopcock; *L*, handle of quick-seating valve, *A*.

was attached a rubber tube with a spring pinchcock, *E*, and a gas sampler, *F*, with its mercury levelling bulb, *G*. A cross-section view of the quick-seating valve, *A*, is shown at *H* with the handle midway between open and closed. The procedure of taking a sample of the alveolar air was as follows: The operator first filled the gas sampler with mercury to above the capillary above the stopcock, *J*, and attached the sampler to the rubber tube with pinchcock, *E*. The stopcock, *J*, was then closed and the spring pinch-

cock, *E*, opened. The operator then watched the breathing as it was recorded on the kymograph. After several respirations had been recorded and it was certain that there was no alteration in the rate or character of the respiration, the operator then gave the signal "blow" to the subject at the end of a normal expiration, at the same time closing the quick-seating valve, *A*, by means of the handle, *L*. When it was seen that the subject had come to the end of a forced expiration, the operator opened the spring pinchcock, *E*, and lowered the mercury levelling bulb, *G*. As soon as the sampler was filled with the alveolar air the operator closed the cock, *J*, signalled the subject "all right," opened the quick-seating valve, *A*, and the subject took a deep inspiration and then breathed as required to recover and continued as normal. During the taking of the sample, 2 to 3 seconds, the subject had held the volume of air in his lungs constant according to instructions which had been given him. The spring pinchcock,

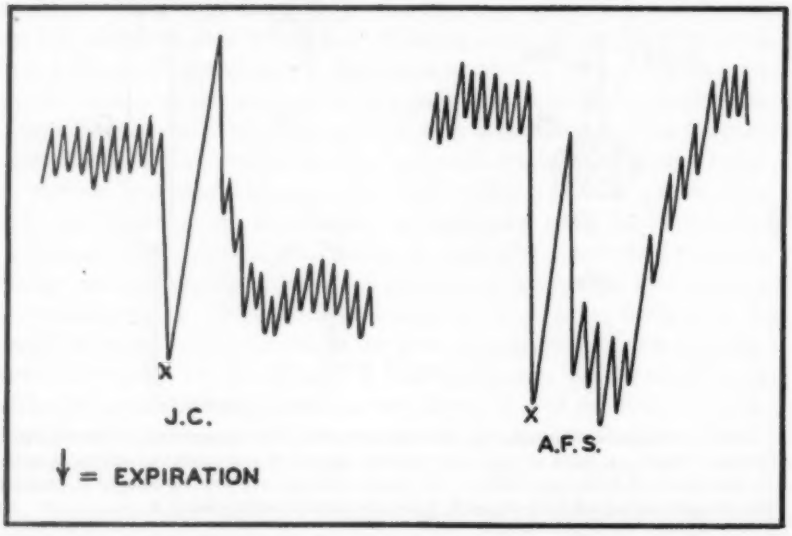

FIG. 2. Graphic record of respiration of subjects J. C. and A. F. S. during the taking of a sample of the alveolar air. Downward stroke is expiration and samples of the alveolar air were drawn at the point, *X*, while the subject kept the volume of the respiratory tract constant.

E, was then closed, the gas sampler detached and another attached in place. The alveolar air samples were analyzed by the Haldane portable gas analysis apparatus. The quick-seating valve prevented inspiration, if attempted, during the taking of the sample, and the whole procedure took

only a few seconds, during which the volume of air in the lungs remained constant. The whole volume of the forced expiration with exception of the amount taken by the sampler was thus a part of the ventilation of the respiratory tract, and was compensated for by the shallowness of the breathing following the collection; thus there was no disturbance in the measurement of the total respiratory exchange. Preliminary trials with this arrangement with two subjects showed that samples could be taken at least every 15 minutes without disturbing the uniformity of the results of the measurements of the total respiratory exchange, and later it was found that samples could be taken every seventh or eighth minute without affecting the total respiratory exchange. A graphic record of the taking of samples of alveolar air with the two subjects used in the experiments is shown in Figure 2. The advantage of this method is that the sample can be obtained as part of the regular respiration experiment. Also, that by our method of measuring the respiratory exchange a graphic record is obtained of the breathing before and at the time the alveolar sample is drawn. It is thus possible to detect changes in the type of breathing due to anticipating the sample or the starting of inhalation before drawing the sample.

The Respiratory Quotient of Expired Air

J. C., a subject whom we have used for many years, was entirely accustomed to the apparatus. The respiratory quotients of this subject for individual periods for each experiment are plotted in Figure 3. As the entire series of experiments was post-absorptive, one would expect that the course of the respiratory quotients during the morning hours would be practically without change and that there would be little variation from period to period, provided the subject sat quietly and was uniformly awake. However, the respiratory quotients with J. C. in the first four fifteen-minute periods were usually at a slightly different level than for the groups of periods succeeding the intermission. In general, with J. C. the 9 to 10 periods after the preliminary hour of 4 periods were slightly lower or slightly higher than the average of the preliminary hour and more uniform than during the first hour. All the experiments were begun immediately after insertion of the mouthpiece but after a half hour's rest, sitting quietly. Even with this subject, who had had long experience with breathing appliance apparatus, a period of adjustment was required before the respiratory exchange became stable. The cause for the shifting of the level of the respiratory quotient after the intermission is not known.

The other subject, A. F. S., had had little previous training. He was familiar with the procedure and had seen many experiments of this type, as he had previously been an assistant in the laboratory.

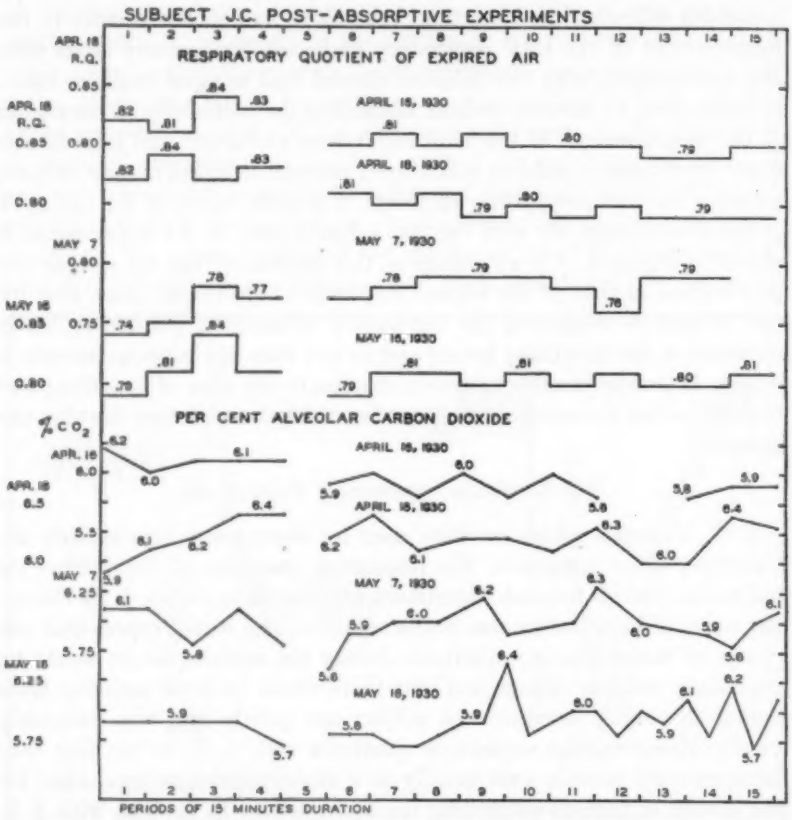
SUBJECT J.C. POST-ABSORPTIVE EXPERIMENTS
RESPIRATORY QUOTIENT OF EXPIRED AIR
PER CENT ALVEOLAR CARBON DIOXIDE
APRIL 16, 1930
MAY 7, 1930
MAY 16, 1930
PERIODS OF 15 MINUTES DURATION

FIG. 3. The respiratory quotient of expired air and the percentage of alveolar carbon dioxide for the individual experiments with J. C. in the post-absorptive state. Period 5 is the interval during which the mouthpiece was taken out corresponding to the later experiments with sugar when the dose was given.

The respiratory quotients for the individual experiments with A. F. S. are plotted in Figure 4. On all three days the initial quotient was low with a gradual rise during the three succeeding periods. The quotients of the first period show strikingly the necessity for a period of preliminary

breathing before beginning the measurements. The period required was longer with A. F. S. than with J. C. The course of the respiratory quotient with A. F. S. was extremely variable, not only during the preliminary hour, but also in the periods following an intermission. As these periods

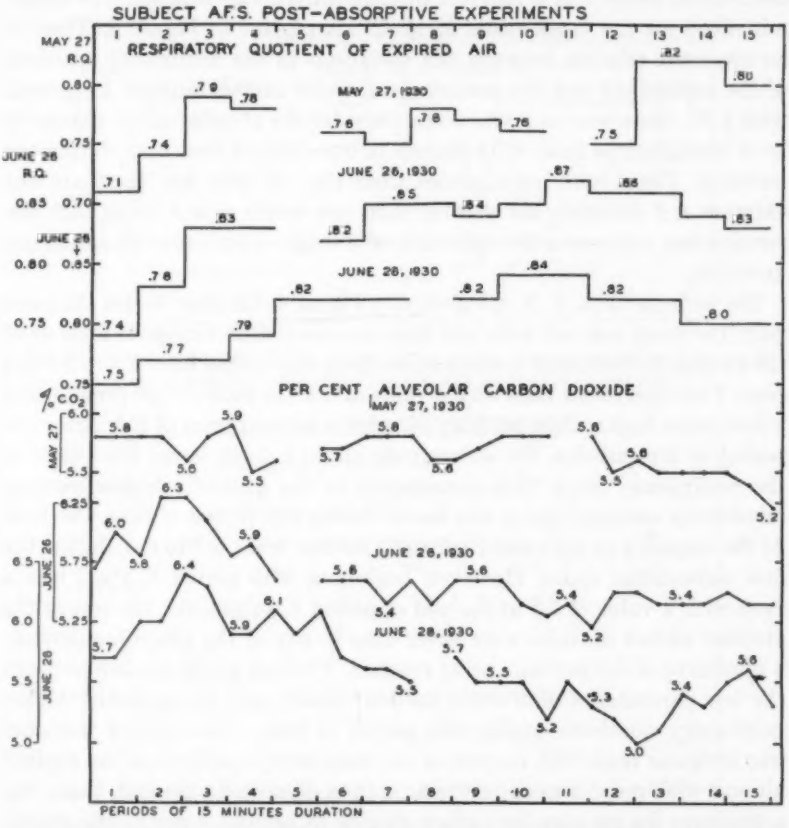

FIG. 4. The respiratory quotient of expired air and the percentage of alveolar carbon dioxide for the individual experiments with A. F. S. in the post-absorptive state.

were all post-absorptive, it is difficult to explain the wide variations, but other subjects (details unpublished) have shown similar characteristics of respiratory exchange. We may regard the first hour as one of adjustment, and the succeeding periods as evidence of instability of the regulatory mechanism with a breathing appliance, or evidences of fatigue.

Percentage of Alveolar Carbon Dioxide

The percentage of alveolar carbon dioxide was determined in parallel with the respiratory exchange at the beginning of the series in this investigation only once during a 15-minute period. Subsequently it was determined every 7 or 8 minutes throughout the experiments. The determinations for the experiments for J. C. are plotted in Figure 3. There is no apparent relation between the variations in the respiratory quotient of the expired air and the percentage alveolar carbon dioxide. In general with J. C. there was no marked tendency for the alveolar air to change in level throughout a total of 14 periods of one-quarter hour each during the morning. There were irregularities from time to time but these are not extreme and probably not greater than one would expect when each determination represents the collection of a single sample and all values are included.

The series with A. F. S. are plotted in Figure 4. On May 27, for the most part, the range was not wide and there was very little change in level until the twelfth to thirteenth periods when there was a drop from 5.8 to 5.5 per cent. The experiment ends with a value of 5.2. On June 26 the preliminary values were higher than on May 27, with a general level of 5.9. After the period of intermission the values were all on a much lower level than in the preliminary hour. This corresponds to the generally higher average respiratory quotient which was found during this period of time. On June 28 the majority of the values were at a normal level, 5.7 to 6.4, during the first consecutive series. However, beginning with period 5, there was a drop with a value of 5.5 at the end of period 7. In general, the rest of the alveolar carbon dioxides were lower than in any of the preceding periods, a minimum of 5.0 per cent being reached. There is a relationship between the low percentages of alveolar carbon dioxide and the generally higher respiratory quotients during this period of time. This subject therefore was irregular both with respect to the respiratory quotient of the expired air and with respect to the alveolar carbon dioxide. In general, there was a tendency for his alveolar carbon dioxide to become lower as the experiments proceeded. This is shown more definitely in the average of all respiratory quotients and the average of the alveolar carbon dioxide which are considered later.

Average Respiratory Quotient of Expired Air

The average respiratory quotient of the expired air for all of the experiments for J. C. and A. F. S. are plotted in Figures 5 and 6. With J. C. there

was practically no change in the average respiratory quotient for 14 periods of 15 minutes each during the morning. For A. F. S. the average of the initial period of the first hour was 0.73, an extremely low value for a subject even in a post-absorptive condition. This represents his adjustment to the apparatus and to the condition of the experiment, although

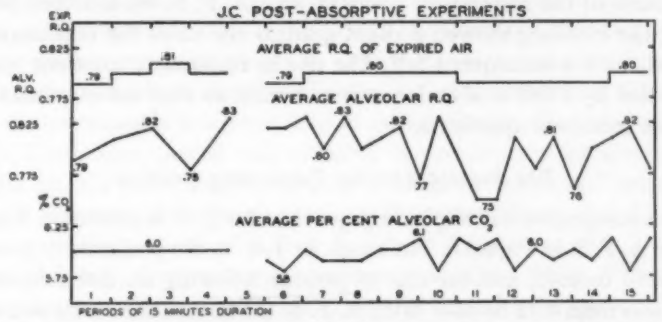

FIG. 5. Average respiratory quotient of expired air, average alveolar respiratory quotient, and average percentage of alveolar carbon dioxide in post-absorptive experiments with J. C.

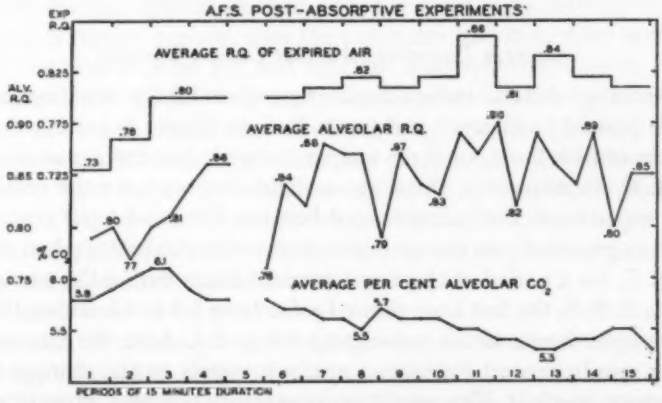

FIG. 6. Average respiratory quotient of expired air, average alveolar respiratory quotient, and average percentage of alveolar carbon dioxide in post-absorptive experiments with A. F. S.

the subject had rested the usual half hour, and measurements were begun at the end of the rest period as soon as the mouthpiece was inserted. The quotient of the next two periods was 0.76 and 0.80. After an intermission of 15 minutes, the average respiratory quotient for A. F. S. showed but little change for 5 successive periods. There was then an abrupt rise to

0.86 with a fall again to 0.81 with varying results to the end of the experiment, when it is again 0.81. The value of 0.86 is the average of 2 experiments instead of 3 for all the other periods. If a value of 0.75 is interpolated for the experiment of May 27, the average of this period (11) becomes 0.82, which is nearly the same as the values in periods 10 and 12. The course of the respiratory quotient with A. F. S. for fourteen periods during the morning showed a slight gradual rise until the maximum was reached, with a subsequent fall. The rise in respiratory quotient was accompanied by a fall in alveolar carbon dioxide so that the quotients were not true metabolic quotients.

The Average Alveolar Respiratory Quotient

The average alveolar respiratory quotient for J. C. is plotted in Figure 5 and for A. F. S. in Figure 6. The range for J. C. in the preliminary hour was from 0.78 to 0.83, and for the 10 periods following an intermission the range was from 0.75 to 0.83. With A. F. S. the fluctuations were somewhat larger as the quotients ranged from 0.76 to 0.90 with a tendency to follow the same course as the quotients of the expired air but at a generally higher level.

Average Percentage Alveolar Carbon Dioxide

The average alveolar carbon dioxide by periods for the experiments with J. C. is plotted in Figure 5 and for A. F. S. in Figure 6. During the preliminary or base-line hour there was no change in the average alveolar air with J. C. Subsequently, there was a slight drop and, for the remainder of the experiment, the values ranged between 5.8 and 6.1 per cent. Thus there was practically no change in the course of the alveolar carbon dioxide with J. C. for a period of three and one-half hours during the morning.

With A. F. S. the first hour showed a rise from 5.8 to 6.1 during the first three quarter-hours with a subsequent fall to 5.8. After the intermission the changes in general correspond nearly inversely to the changes in the respiratory quotient. The maximum respiratory quotient came in period 11 when the alveolar carbon dioxide was 5.5. Again a rise in quotient occurred in period 13 with a concurrent fall in alveolar carbon dioxide. With A. F. S. both the respiratory quotient and the alveolar carbon dioxide showed changes in the course of the morning which were not consistent with what one would expect in a series of periods in the post-absorptive condition. There is no theoretical reason for a rise in the respiratory quotient in the course of three hours and a half in the morning or for a fall in

the alveolar carbon dioxide when a subject is in a post-absorptive condition after an ordinary mixed diet.

Relation of Alveolar Respiratory Quotients to Alveolar Carbon Dioxide

If the sampling of the alveolar air is made incorrectly, the resultant carbon dioxide content of the alveolar air may be too low or too high according to whether there was a dilution of the alveolar air by too deep an inspiration or a retention of carbon dioxide due to too shallow breathing at the instant of taking samples. If there is over-ventilation or the respiratory center responds to a lower level of carbon dioxide, the carbon dioxide will be lower than normal and probably the respiratory quotient of the alveolar air will be too high because it is easier to "wash out" carbon dioxide by over-ventilation than to increase the oxygen absorption. Although the range of the per cent of carbon dioxide of alveolar air with J. C. was narrow (5.7–6.1), there were some values outside these limits. What is the relation between the fluctuations in the percentage alveolar carbon dioxide and the alveolar respiratory quotient? If the above reasoning is correct, when the carbon dioxide content of alveolar air is low, the alveolar respiratory quotient should be high, and vice versa. This assumes that the sampling is correctly made, that the expiration had been deep enough to obtain the real alveolar air, and that the analyses were correct. At the beginning of this work it was the intention to obtain the carbon dioxide only, and owing to the pressure of other work at the same time, duplicate analyses were not made of the samples obtained from the alveolar air. Whenever there was a value which was markedly aberrant the analysis was repeated. The object of the study was to obtain the general trend of the alveolar air, particularly the carbon dioxide in relation to the simultaneous changes in respiratory quotient of the expired air.

In Figure 7 are plotted the relationships between the alveolar carbon dioxide and the alveolar respiratory quotient for the two subjects. The range in alveolar respiratory quotients with J. C. was not wide, yet in spite of this there is a definite relationship between the respiratory quotient and the alveolar carbon dioxide content. As the carbon dioxide content falls, the respiratory quotient rises. This finding probably represents the slight variations in depth of respiration immediately preceding the taking of the sample. It must be recalled that only one sample was taken and this was at the end of a normal expiration. It is surprising that even with a narrow range of carbon dioxide content the relationship is evident.

With A. F. S., the values were scattered, but there was a tendency to a

relationship between the alveolar respiratory quotients and the alveolar carbon dioxide content. The relationship is not so marked as with J. C. This is in line with the variability of the respiratory center of this subject. There is a lack of uniform response and consequently a relationship between various factors in breathing will not be so constant with this subject

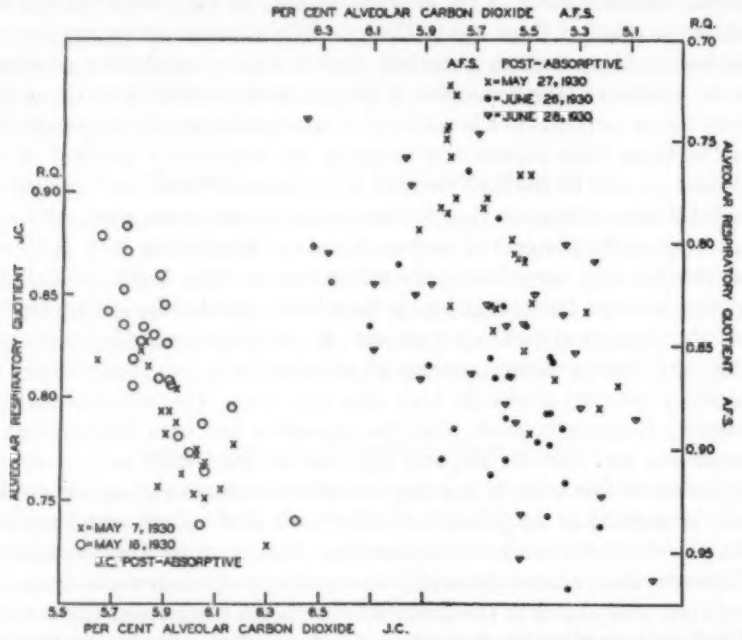

FIG. 7. Diagram showing relationship between percentage alveolar carbon dioxide and alveolar respiratory quotient for J. C. and A. F. S. in post-absorptive experiments. The experiments with A. F. S. are plotted in an inverse manner to those of J. C.

as with J. C., with whom all of the factors of ventilation and respiratory exchange were comparatively more consistent (unpublished material).

Relation between the Alveolar Respiratory Quotient and the Alveolar Oxygen Deficit

The respiratory quotient of the alveolar air is calculated in the same manner as that of the expired air, that is, the increase of carbon dioxide is divided by the oxygen deficit of the alveolar air. The relation between the alveolar respiratory quotient and the alveolar carbon dioxide is more apparent in the extremes than in the majority of the measurements. There-

fore one would expect from the mathematical derivation of the respiratory quotient that the oxygen deficit would bear an inverse relationship to the respiratory quotient just as it does in the main in the expired air when a subject is at rest (Carpenter and Fox, 2). It can be argued that with a nearly constant alveolar carbon dioxide percentage the alveolar respiratory quotient must vary with the alveolar oxygen deficit even if the analyses

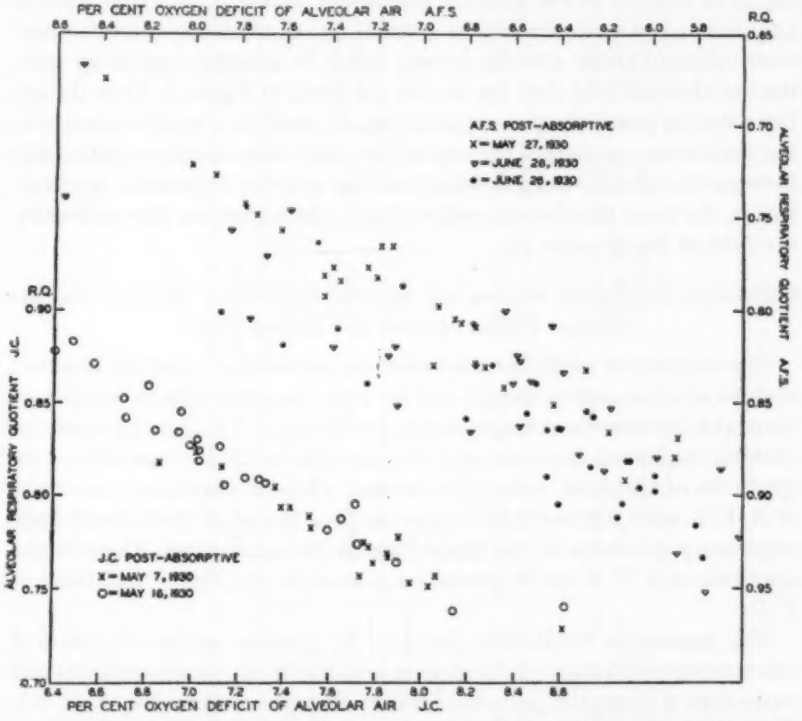
PER CENT OXYGEN DEFICIT OF ALVEOLAR AIR A.F.S.
R.Q.
A.F.S. POST-ABSORPTIVE
X = MAY 27, 1930
▽ = JUNE 26, 1930
• = JUNE 28, 1930
ALVEOLAR RESPIRATORY QUOTIENT A.F.S.
ALVEOLAR RESPIRATORY QUOTIENT J.C.
J.C. POST-ABSORPTIVE
X = MAY 7, 1930
O = MAY 16, 1930
PER CENT OXYGEN DEFICIT OF ALVEOLAR AIR J.C.

FIG. 8. Diagram showing relationship between the percentage alveolar oxygen deficit and the alveolar respiratory quotient for J. C. and A. F. S. in post-absorptive experiments. The experiments with A. F. S. are plotted in an inverse manner to those of J. C.

were wrong. This cannot be denied in our results because only one analysis was made of each sample, but the apparatus were standardized each day with outdoor air, and repetition of doubtful results usually gave agreeing results.

The percentages of alveolar oxygen deficit in relation to the alveolar respiratory quotients for J. C. and A. F. S. have been plotted in Figure 8.

In spite of the narrow range of the majority of the respiratory quotients, there is a very marked relationship between the alveolar oxygen deficit and the alveolar respiratory quotient in both experiments with J. C. Therefore with J. C. the alveolar respiratory quotients varied inversely as the alveolar oxygen deficits in the post-absorptive state.

With A. F. S. there was a noticeable variation in the alveolar oxygen deficit in relation to the alveolar respiratory quotient, although there is unquestionably a relationship as a whole. In all three experiments there were values of either alveolar oxygen deficit or alveolar respiratory quotient so aberrant that they are outside the limits of Figure 8. Even though there was no metabolic process which would result in a marked change in the respiratory quotient of the expired air, there was a definite relationship between the alveolar oxygen deficit and the alveolar respiratory quotient, that is, the lower the alveolar oxygen deficit, the higher was the respiratory quotient of the alveolar air.

Correlation Coefficients between the Alveolar Respiratory Quotient and the Alveolar Carbon Dioxide and Oxygen Deficit

The correlation coefficients between the alveolar respiratory quotient and the alveolar carbon dioxide and between the alveolar respiratory quotient and the alveolar oxygen deficit are given in Table I. The average alveolar respiratory quotients of J. C. were close to the average respiratory quotients of the same dates. The average alveolar respiratory quotients of A. F. S. are 0.039 and 0.033 higher on June 26 and 28 than the average respiratory quotients of the expired air on the same dates. The alveolar quotients of A. F. S. are in general more variable and higher than those of J. C.

The correlation coefficients between the alveolar carbon dioxide and the alveolar respiratory quotient were negative in all the experiments and more than 6 times the probable error in all but one (June 28, A. F. S.). There is therefore a definite relationship between the alveolar carbon dioxide and the alveolar respiratory quotient, that is, the higher the alveolar carbon dioxide, the lower the respiratory quotient. The relationship shown in Figure 7 therefore becomes much more evident when expressed mathematically. It is surprising that the relationship is so marked with J. C. when the relatively narrow ranges of both the alveolar carbon dioxide and respiratory quotient are considered. The lower correlation coefficients between these factors for A. F. S. are in part due to the irregularities in breathing and irregularities in the alveolar respiratory quotients.

TABLE I

CORRELATION COEFFICIENTS BETWEEN THE ALVEOLAR RESPIRATORY QUOTIENTS AND THE ALVEOLAR CARBON DIOXIDE AND OXYGEN DEFICIT IN NO-DOSE EXPERIMENTS

Subject and date	Number of determinations	Alveolar R.Q.		Alveolar CO_2		Alveolar O_2 deficit		Correlation coefficients			
		Average	S.D.	Average	S.D.	Average	S.D.	Alveolar R.Q. and alveolar CO_2		Alveolar R.Q. and alveolar O_2 deficit	
1930				%		%		r	P.E.$_r$	r	P.E.$_r$
J.C.											
May 7	20	0.780±0.022	0.026	5.97±0.11	0.14	7.63±0.34	0.42	−0.801±0.054		−0.964±0.011	
May 16	25	0.817±0.031	0.038	5.89±0.12	0.16	7.20±0.42	0.52	−0.808±0.047		−0.967±0.009	
A. F. S.											
May 27	31	0.786±0.049	0.064	5.64±0.16	0.19	7.20±0.62	0.80	−0.631±0.073		−0.979±0.005	
June 26	30	0.873±0.043	0.056	5.61±0.21	0.27	6.43±0.49	0.63	−0.537±0.088		−0.894±0.025	
June 28	30	0.839±0.047	0.063	5.63±0.27	0.33	6.73±0.63	0.80	−0.518±0.090		−0.906±0.022	

The correlation coefficients between the alveolar respiratory quotients and the alveolar oxygen deficits were all negative and very marked. It should be remarked that analytical errors in the determination of the oxygen content of the alveolar air would bring about a good correlation between the respiratory quotient and the oxygen deficit when the range of the alveolar carbon dioxide is narrow, for mathematically it must follow that as the oxygen deficit falls off the respiratory quotient rises. A good correlation between the oxygen deficit and the respiratory quotient should never be taken as an index of the reliability of the analyses, as this must rest on other grounds.

These highly negative coefficients are indication of variations in the alveolar quotients due to metabolic variations in the respiratory exchanges in the same manner as the reciprocal relationships of the respiratory quotient and oxygen deficit of the expired air. It is recognized that not all of the variations with A. F. S. were truly metabolic variations, but part of them were due to shifts in the respiratory quotients accompanied by shifts in the general level of the alveolar carbon dioxide.

Summary

The respiratory exchange of two human subjects was measured on 3 and 4 days for 4 consecutive 15-minute periods, and then for 9 or 10 consecutive 15-minute periods in the post-absorptive condition, in a sitting position, by means of an open-circuit respiration apparatus with mouthpiece as a breathing appliance. The alveolar air was sampled at least every 15 minutes without interrupting the measurements of the respiratory exchange, by means of an arrangement described in detail.

With one trained subject the average respiratory quotient and the alveolar carbon dioxide did not change significantly in the course of a total of three and one-half hours. With one untrained subject both the respiratory quotient and the alveolar carbon dioxide varied, usually in opposite directions during the same length of time. The alveolar respiratory quotients of both subjects tended to run parallel with the respiratory quotients of the total expired air.

There was a tendency to an inverse relationship between the alveolar carbon dioxide and the alveolar respiratory quotients which was more marked in the extreme values, and with the trained subject than with the untrained subject and was due either to slight variations in ventilation at the time of taking of the alveolar air samples or to variations in the sensitivity of the respiratory center.

There was a marked negative correlation between the alveolar respiratory quotient and the percentage alveolar oxygen deficit.

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THE EFFECT OF GLUCOSE AND OF FRUCTOSE ON THE HUMAN RESPIRATORY QUOTIENT AND ALVEOLAR AIR*

By

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THE effect of the ingestion of glucose on the respiratory exchange of animals and man has been determined repeatedly and a well established fact in the physiology of metabolism is that the respiratory quotient usually rises when the sugar is ingested. It is logical to assume that the increase in respiratory quotient is the result of the increase in the carbohydrate combustion because glucose is a constituent of many nutrients. However, that organic acids may be formed in the intermediary metabolism is by no means excluded because of the repeated results which have been obtained, or because it seems logical that there should be an increase in the carbohydrate combustion after the ingestion of glucose.

Practically all of the investigations with human subjects on the effect of the ingestion of fructose on the respiratory quotient in short periods show that there is a marked and prompt rise in the respiratory quotient. As long as this respiratory quotient was not over unity, it has been considered by many that there was an increase in the catabolism of carbohydrate as a result of the ingestion of fructose. In a study reported previously from this laboratory (Carpenter and Fox, 3), the effects of small quantities, 5 to 50 grams, were studied with respect to the respiratory quotient and the total metabolism. It was found that a marked rise occurred whether a small or large quantity was used and the main variation was the height to which the respiratory quotient rose and the length of time it remained above the pre-ingestion level. As the respiratory quotient will rise to over unity when larger quantities are given, it was postulated that the reaction was one of transformation of carbohydrate to fat, and that this took place regardless of the quantity ingested. No studies have been carried out in which the respiratory quotient and simultaneous de-

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terminations of the alveolar air directly have been obtained. It is of importance to know whether the changes in respiratory quotient due to glucose and to fructose are the results of metabolic processes only, or whether they are the results of combustion plus the influence of acids which may be formed in the intermediary metabolism of the sugars and lead to the expulsion of carbon dioxide from the blood with a fall of the carbon dioxide content of the blood.

The possibility of the formation of organic acids and of changes in the reaction of the blood in the intermediary metabolism of glucose and of fructose and their relation to the respiratory exchange have been studied in several ways. Taistra (12) determined the CO_2-combining power of the blood plasma of a dog in the 4 hours after the ingestion of 50 grams of glucose and found no significant change as compared with the pre-ingestion values. Moraczewski and Lindner (9) found a greater excretion of lactic acid in the urine of patients after the intravenous injection of 50 grams of fructose in 50 cc. of distilled water than after injection of glucose. Weiss and Klein (13) determined with narcotized rabbits the respiratory exchange and carbon-dioxide content of the blood at intervals after the ingestion by stomach tube of 15 to 30 grams of glucose and found no significant alteration in the carbon dioxide of the blood. Douglas and Priestley (5) found that the alveolar carbon dioxide remained practically unchanged after the ingestion of 75 and 80 grams of cane sugar. Gigon (6) measured the pH of the blood of men after the ingestion of 100 grams of glucose and found a maximum fall from 7.3 to 7.0 in 45 minutes. He also obtained similar results with rabbits. He found a lowering of the pH of the blood in man equivalent to 0.3 after the ingestion of 100 grams of fructose in 300 cc. of water. Lanyi (8) found that the administration of dextrose to dogs or man did not result in an increase of lactic acid in the blood, but the ingestion of fructose was followed by an increase in the lactic acid content of the body lasting 3 hours and returning to normal after 6 hours. Katayama (7) found an increase in lactic acid of the blood simultaneous with the hyperglycemia which took place after the ingestion of 1.75 grams of dextrose per kilogram of body weight in 22 normal and diseased subjects. Oppenheimer (10) found no increase in lactic acid in the blood of normal persons or of patients with diseases of the liver when 50 grams of glucose or of fructose in 300 cc. of water were taken by mouth. Campbell and Maltby (1) found no alteration in the CO_2-combining power and lactic acid content of the blood in man as the result of the ingestion of either 100 grams of glucose or of maltose, but the ingestion of 100 grams of fruc-

tose was followed by falls of 1 to 10 volumes per cent with simultaneous increases in the lactic acid content of the blood. Rose, Giragossintz, and Kirstein (11) injected 25 grams of fructose or glucose in 125 cc. of water into the small intestine and determined the lactic acid in the portal, the hepatic and the femoral veins and femoral artery. There was an increase of 50 to 100 per cent in the lactic acid content of the portal vein after injection of fructose but no significant change after glucose. The lactic acid disappeared in the liver as the hepatic vein showed but slight increase in lactic acid content. Wierzuchowski and Laniewski (14) determined the lactic acid content of the blood and urine in fasting dogs before and after the intravenous injection of 2 grams per kilo. per hour of solutions of glucose, fructose, and galactose for 2 hours. All three sugars brought about an increase in the lactic acid content of the blood with fructose producing the greatest increment. The rises of lactic acid in the blood were accompanied by an increased lactic acid elimination in the urine and by reciprocal changes in the CO_2-combining power of the blood.

Most of the studies reported in the literature indicate that an increase in the lactic acid content of the body results from the introduction of fructose. However, many of the investigations were made by the intravenous injection of the sugar solution and it is not improbable that the character of the metabolism of a substance differs according to the route by which it is introduced. In a number of studies, the doses were large and overloading may have led to abnormal metabolism. In some studies, the ingestion of glucose also brought about similar changes in the blood reaction and lactic acid content although not always to the same degree as was brought about by the introduction of fructose.

This publication gives the results of another method of attack on the problem of the metabolism of glucose and of fructose, that is, a comparison of the total respiratory quotients with the composition of alveolar air at intervals after the ingestion of the sugars.

The Respiratory Exchange and Alveolar Air as Affected by the Ingestion of 25 grams of Glucose or of Fructose

Method of study.—The subjects and procedures used were the same as those described in the preceding paper. The respiratory exchange was measured in 4 15-minute periods with the subjects in the post-absorptive condition. There was then a rest period of about 15 minutes, when the subject drank a solution of 25 grams of glucose or fructose in 200 cc. of water at 37°C., and immediately inserted the mouthpiece of the respiration

apparatus. Measurements were begun at once and continued uninterruptedly for 10 successive 15-minute periods. The amount of glucose or fructose taken had been found in previous studies to cause definite rises in the respiratory quotient and metabolism (Carpenter and Fox, 2, 3) with a return to nearly pre-ingestion values in two and one-half hours. The alveolar air samples were collected once or twice each 15-minute period during the post-absorptive portions of the experiments and at the first and eighth minute of each period after the ingestion of the sugars.

The Respiratory Quotient before and after the Ingestion of 25 grams of Glucose or Fructose

The experiments reported here were made primarily for the purpose of comparing the respiratory quotients as affected by the ingestion of 25 grams of glucose or fructose with the alveolar air collected at intervals. The respiratory quotients for J. C. are plotted in Figures 1 and 2. The quotients in the 4 post-absorptive or base-line periods show the same tendency to low values in the first periods with slightly higher values in the succeeding periods, as in the series in the preceding paper (see p. 41). After the ingestion of glucose, the quotients reached a maximum in the ninth 15-minute period, and were sustained at a high level until the tenth to eleventh period of the experiment. They subsequently fell but even at the end of two and one-half hours after the sugar ingestion they did not reach the post-absorptive quotient in all the experiments.

The effect of the fructose was similar to that which has been found in previous investigations, the maximum occurring usually in the third quarter-hour after ingestion, with a return to nearly normal at the end of two and one-half hours. Attention is called to the high pre-ingestion quotients of May 12, accompanied by the much greater maximum height of the quotient after fructose as compared with the course of the quotients in the other three experiments with the same subject.

The respiratory quotients of A. F. S. before and after the ingestion of glucose and fructose are shown in Figures 3 and 4. The first preliminary post-absorptive quotients were lower than usually found in a normal subject as there are several quotients below 0.75. In the eighth period of the glucose experiments (third after ingestion) there was a rise in the respiratory quotient, but this was not uniform.

In the first two periods of the experiments after the ingestion of fructose, there was but little effect on the respiratory quotient due to the ingestion of sugar, in the third period there was a marked rise, and in the fourth

period occurred the maximum respiratory quotient after the ingestion of fructose. The period of maximum rise was slightly later with this subject than with J. C. and the uniformity in the rise in the quotient was not so great as with J. C.

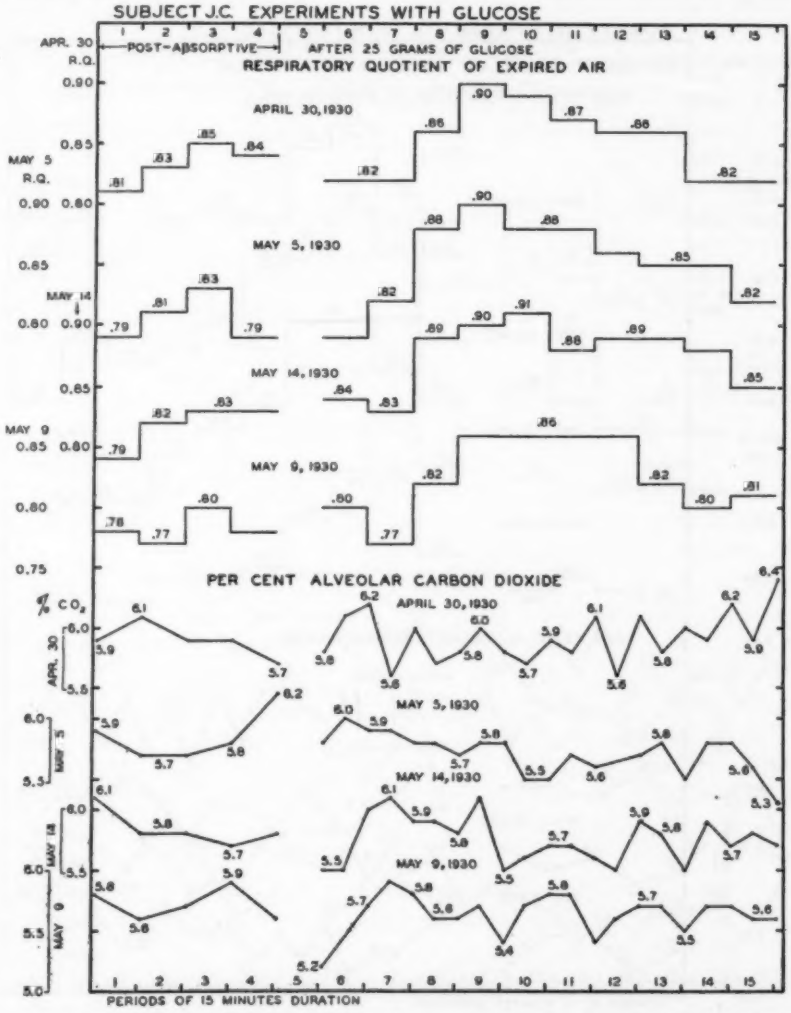

FIG. 1. Respiratory quotient of expired air and percentage alveolar carbon dioxide in individual experiments with J. C. before and after the ingestion of 25 grams of glucose.

Percentage of Alveolar Carbon Dioxide as Affected by the Ingestion of 25 grams of Glucose or Fructose

The individual determinations of percentage of alveolar carbon dioxide for J. C. before and after the ingestion of 25 grams of glucose are plotted

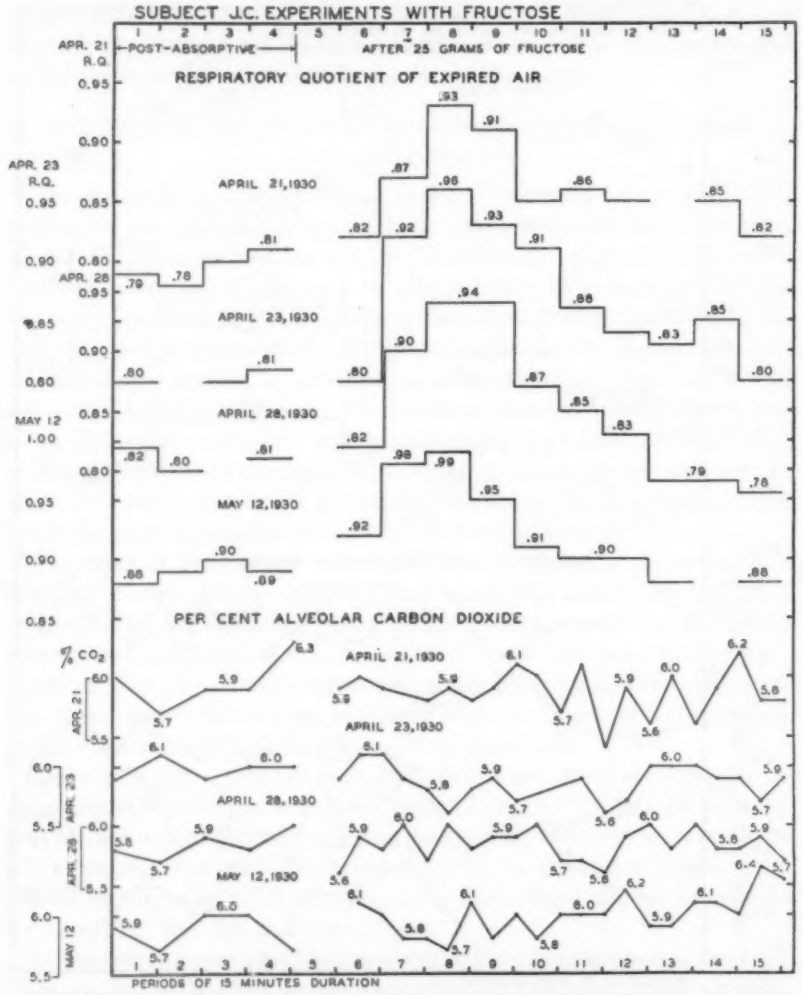

FIG. 2. The respiratory quotient of the expired air and the percentage alveolar carbon dioxide in experiments with J. C. before and after the ingestion of 25 grams of fructose.

in Figure 1 and for the experiments with fructose in Figure 2. The values in the four 15-minute periods before ingestion of the sugars in both groups varied from 5.6 to 6.3 in the extremes, although the majority of the values were within narrower limits, 5.7 to 6.0 per cent, and there is no tendency

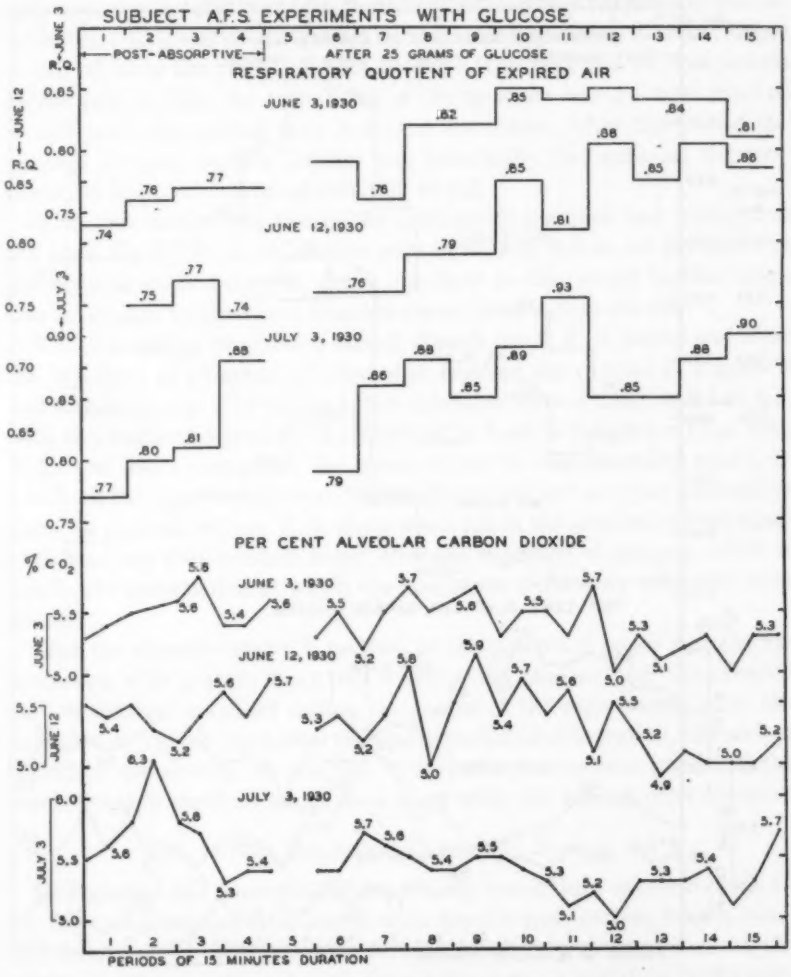

FIG. 3. Respiratory quotient of expired air and percentage of alveolar carbon dioxide in the individual experiments with A. F. S. before and after the ingestion of 25 grams of glucose.

to a rise or a fall. In three out of four experiments with glucose there was no definite change as the result of the ingestion of the sugar.

There was but little significant change in the alveolar carbon dioxide throughout the two and three-quarter hours after fructose was given, al-

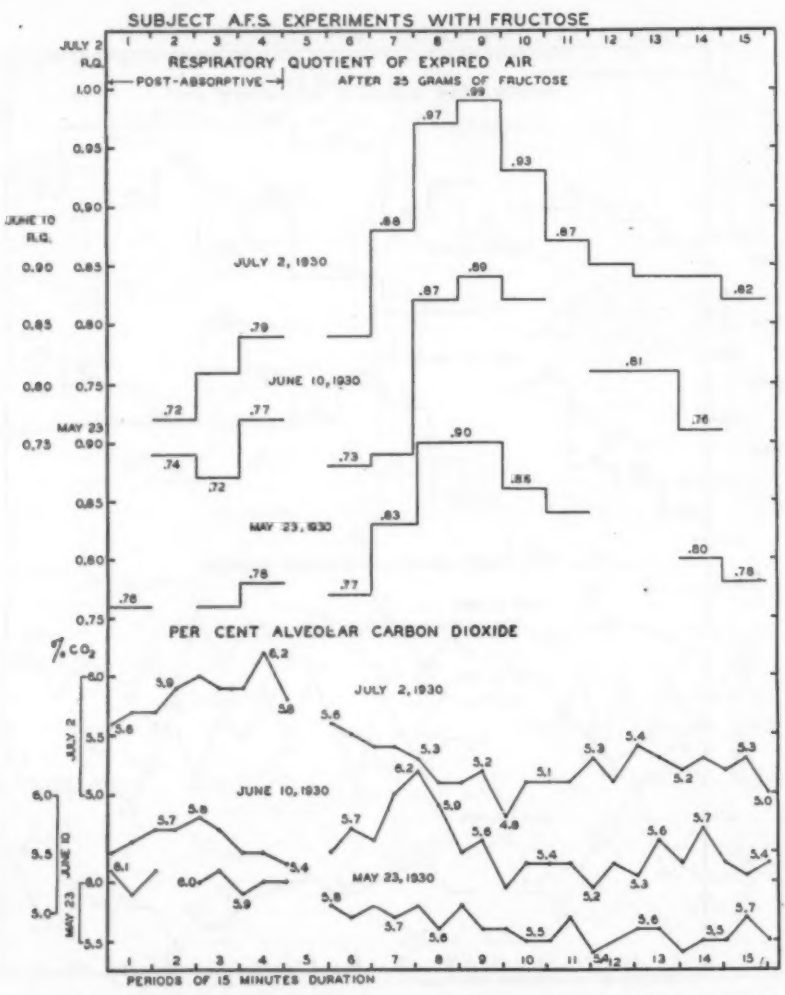

FIG. 4. The respiratory quotient of the expired air and the percentage alveolar carbon dioxide in experiments with A. F. S. before and after the ingestion of 25 grams of fructose.

though there were fluctuations from one determination to another, with occasionally marked variations. Curiously, in the twelfth period or one and three-quarter hours after ingestion, there was a particularly low value ranging from 5.4 to 5.6 in three of the experiments out of four, but this was the only point of time during the whole series in which there was a noticeable deviation from the normal level in practically the majority of the experiments. This was not the period in which the respiratory quotient began to fall off after the peak had been reached. The peak was several periods before this, so that the rate of fall of the quotient was no more marked in this particular period than in any of the others. After this period the average alveolar carbon dioxide was practically the same as the pre-ingestion level, with a range from 5.9 to 6.0.

With this subject the rise in the respiratory quotient and subsequent fall after the ingestion of fructose were in no way due to an alteration in the alveolar carbon dioxide, and a corollary to this would be that there was no change in the blood bicarbonate or total carbon dioxide.

The percentages of alveolar carbon dioxide for A. F. S. before and after the ingestion of 25 grams of glucose or fructose are plotted in Figures 3 and 4 respectively. The values before ingestion show a range of 5.2 to 6.3, with the majority between 5.4 and 5.7. The level is thus lower than with J. C. and lower than with the same subject in the preceding study, in which all the experiments were made with the subject in a post-absorptive state. In general, with A. F. S. there was a fall in the alveolar carbon dioxide about one and one-half hours after the ingestion of glucose, which is nearly the same period at which the maximum respiratory quotients were found.

Like the experiments with no dose in the preceding paper and the experiments with glucose there was a noticeable tendency for the alveolar carbon dioxide to fall off during the course of the experiments after the ingestion of fructose, and after the fourth period after ingestion, the carbon dioxide of the alveolar air was low. This is one-quarter hour later than the marked rise in quotient which took place with this subject after fructose.

The Average Respiratory Quotient of Expired Air

In Figures 5 and 6 are plotted the average respiratory quotients of J. C. for the two groups of experiments with glucose and fructose for the base-line hour and for 10 15-minute periods succeeding the ingestion of the sugars. In the experiments with glucose there was a variation in the quotient of expired air of 0.040 in the 4 base-line periods. After the ingestion

of glucose there was no apparent effect for one half-hour. Then there was a marked rise, so that within the next half-hour the peak was reached. Beginning one and one-half hours after the ingestion there was a gradual fall, so that one hour later the quotient was 0.015 above the average base-line respiratory quotient. The maximum rise from the base line was 0.08

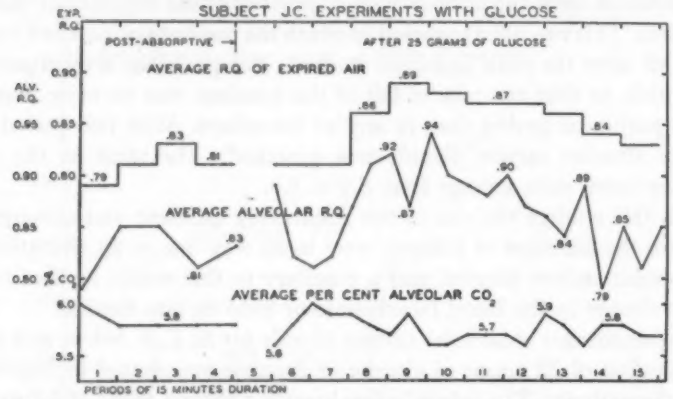

FIG. 5. Average respiratory quotient of expired air, average alveolar respiratory quotient, and average percentage alveolar carbon dioxide in experiments with J. C. before and after the ingestion of 25 grams of glucose.

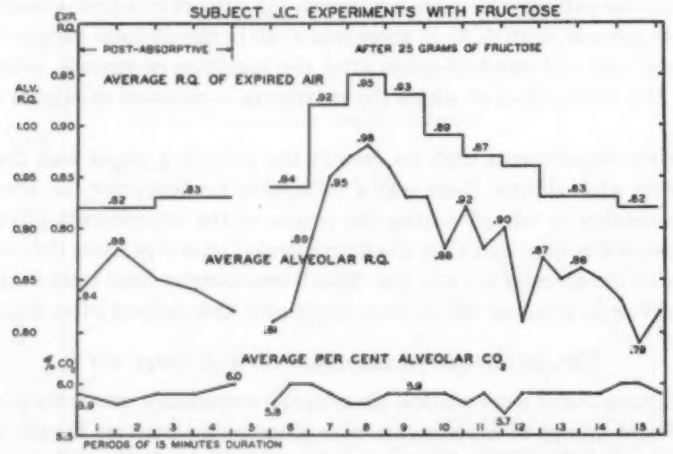

FIG. 6. Average respiratory quotient of expired air, average alveolar respiratory quotient, and average percentage of alveolar carbon dioxide in experiments with J. C. before and after the ingestion of 25 grams of fructose.

and there was a marked increase above the base-line level for one and one-half hours. In the experiments with fructose, the base-line or pre-ingestion periods of J. C. had a nearly uniform average respiratory quotient of 0.82 and 0.83. After the ingestion of the sugar, there was a sharp rise to a maximum of 0.95 in the eighth period of the group or the third quarter-hour after ingestion. From there on until the end of the experiments the average respiratory quotients fell to 0.82, the pre-ingestion level.

The average respiratory quotients of the expired air of A. F. S. for the two groups of experiments with glucose and fructose are shown in Figures 7 and 8. In the group with glucose there was a steady rise of 0.040 in the

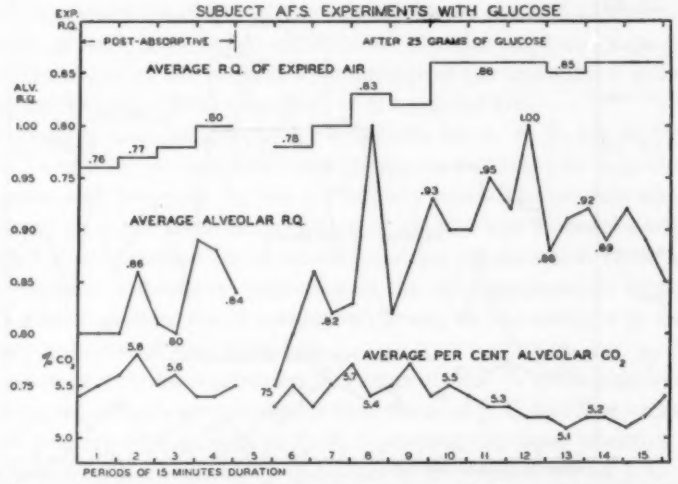

FIG. 7. Average respiratory quotient of expired air, average alveolar respiratory quotient, and average percentage of alveolar carbon dioxide in experiments with A. F. S. before and after the ingestion of 25 grams of glucose.

4 base-line periods before ingestion. Immediately after the ingestion there was but a slight change until the third quarter-hour in which the rise was 0.05 above the average base line. During the next half hour the quotient rose so that it was 0.08 above the base line. This higher level lasted for practically the rest of the experiment, that is, during a total of one and one-half hours. The rise in the respiratory quotient after the ingestion of glucose with both subjects, therefore, was about of the same order, although the initial level is 0.035 lower with A. F. S. than with J. C. The difference between the two subjects was that with A. F. S. there was not

a fall shortly after the peak was reached as there was with J. C. The course of the quotient after the ingestion of glucose with J. C. was more nearly like that in previous studies than was that of A. F. S.

In the group of experiments with fructose, the average respiratory quotients showed a rise in the 3 pre-ingestion periods from 0.74 to 0.78. After the ingestion of 25 grams of the sugar there was a rise from 0.77 to 0.92 in the ninth period or the fourth quarter-hour after ingestion. From there on until the end of the experiment, there was a fall to 0.80, a value 0.04 above

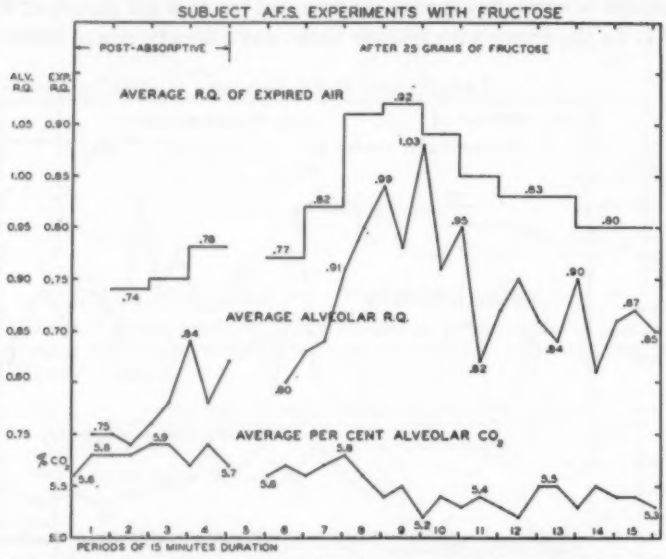

FIG. 8. Average respiratory quotient of expired air, average alveolar respiratory quotient, and average percentage of alveolar carbon dioxide in experiments with A. F. S. before and after the ingestion of 25 grams of fructose.

the average pre-ingestion level of 0.76. Thus there was a greater rise in the respiratory quotient after fructose with A. F. S. than with J. C. but part of this rise, as explained later, was due to a fall of the alveolar carbon dioxide with an accompanying over-ventilation or extra elimination of carbon dioxide over that produced as the result of the ingestion of the fructose.

Average Alveolar Respiratory Quotient

The average alveolar respiratory quotient of J. C. is shown in Figures 5 and 6 for the groups of experiments with glucose and fructose respectively.

After the ingestion of glucose there were marked variations from one period to another, but the smoothed curve which could be drawn from the determinations is somewhat of the same shape as the respiratory quotient of the expired air. Its peak, however, is somewhat higher, averaging about 0.92 and, in general, the alveolar respiratory quotient will average slightly higher all through the experiments than the respiratory quotient of the expired air.

After the ingestion of fructose there was a sharp rise in the first three periods to a maximum of 0.98 in the third period after ingestion, which is 0.03 higher than the respiratory quotient of the expired air in the same period. From there on there was an irregular fall to nearly the same level as the respiratory quotients of the expired air at the end of the experiments. In general, a smoothed curve of the alveolar respiratory quotients follows the course of the respiratory quotients of the expired air, although at a generally higher level than that of the expired air.

The average alveolar respiratory quotients for A. F. S. are plotted in Figures 7 and 8 for the experiments with glucose and fructose respectively. They were very irregular for the preliminary hour. The average alveolar respiratory quotient after the ingestion of glucose was extraordinarily irregular, but a smoothed curve taken from the points which show a rise in the respiratory quotient, beginning at the third one-quarter hour (period 7) after ingestion would correspond nearly to the changes in the respiratory quotient of the expired air.

The average alveolar respiratory quotients of A. F. S. after the ingestion of fructose were much more irregular than those of J. C. but as a whole the alveolar respiratory quotients of A. F. S. showed the same tendency to a parallelism with the respiratory quotients of the expired air as J. C.

In general one can conclude from the respiratory quotient of the alveolar air that its course, when taken as a whole, corresponds somewhat to the course of the respiratory quotient of the expired air, but that with both subjects the alveolar quotient is slightly higher.

Average Percentage Alveolar Carbon Dioxide

The average percentage alveolar carbon dioxide in the two groups of experiments with J. C. is plotted in Figures 5 and 6. During the preliminary base-line hour the maximum difference was 0.20 per cent. At the beginning of the period after the ingestion of glucose, the alveolar carbon dioxide on the average is lower than during the base-line hour, but during the next 15 minutes rises to a level that, on the average, is about the same as that

during the base-line hour. There was but slight alteration or change in direction of the alveolar carbon dioxide percentage. As judged by the percentage of carbon dioxide in the alveolar air, there is no indication that the respiratory quotient is other than a metabolic one, that is, that the rise and fall in the respiratory quotient represent an increase and a decrease in the proportion of carbohydrates catabolized.

The course of the alveolar carbon dioxide in the experiments with fructose was for the most part regular throughout the whole series of periods. The range was 0.30 in the 21 points after ingestion of fructose. There is no definite indication of a change in the course of the alveolar carbon dioxide after the ingestion of fructose which is greater than the changes in the pre-ingestion periods.

The average percentage alveolar carbon dioxide in the two groups of experiments with A. F. S. is plotted in Figures 7 and 8. The average percentage during the base-line hour was nearly uniform. Beginning with the 5th 15-minute period after the ingestion of glucose (period 10), the percentages, on the average, were lower by about 0.40 and tended to fall off gradually. Thus the last part of the change in respiratory quotient of expired air due to the ingestion of glucose with this subject is accompanied by a change in the alveolar carbon dioxide percentage. Whether this is an effect due to glucose or whether it is the result of the natural course of the alveolar air of this subject during the morning hours cannot be stated.

In the third period after ingestion of fructose (eighth of the experiments) there was a definite drop in the average alveolar carbon dioxide and during the remainder of the experiments it averaged between 5.5 and 5.2 per cent, thus definitely lower than the range of the pre-ingestion level (5.6–5.9). The ingestion of 25 grams of fructose with A. F. S. was therefore accompanied by a significant lowering of the alveolar carbon dioxide from the period of the maximum respiratory quotient until the end of the experiments. Therefore not all of the rise in the respiratory quotient of the expired air with A. F. S. as the result of ingestion of fructose can be ascribed to a metabolism unaccompanied by a change in level of the alveolar carbon dioxide.

So far as J. C. is concerned, it can be stated definitely that the effect of the ingestion of fructose was not to change in any way the alveolar carbon dioxide, and one would be warranted by this standard alone to draw the conclusion that the respiratory quotient after the ingestion of fructose with this subject represented the metabolic changes only and not the effect in part or wholly of the shift in equilibrium of the blood. On the contrary,

with A. F. S. one would be justified in drawing a conclusion that the effect of fructose was to cause the alveolar carbon dioxide to fall and, therefore, there had been an elimination of more carbon dioxide than was produced as the result of the ingestion of fructose. This subject showed, however, the same sort of results when experiments were carried out the same length of time and no dose was given. Consequently it is a question whether a fall of the alveolar carbon dioxide is the result of the ingestion of fructose or whether it is a normal characteristic of this subject regardless of what material is ingested. Therefore, the respiratory quotient studied with a subject such as this cannot be of the same significance as quotients obtained from subjects who, in post-absorptive condition with no dose, show a uniform alveolar carbon dioxide from hour to hour throughout the period of four to five hours.

Relationship between Alveolar Respiratory Quotients and Alveolar Carbon Dioxide

The relationship between the alveolar respiratory quotient and the alveolar carbon dioxide is plotted for J. C. and A. F. S. in Figure 9. The alveolar respiratory quotients of J. C., for the most part, ranged between 0.80 and 0.92, but the alveolar carbon dioxide for the same limits of respiratory quotients ranged between only 5.5 and 5.9 per cent. The extremes are a low respiratory quotient of 0.765 with an alveolar carbon dioxide of 6.18 per cent and a respiratory quotient of 0.97 for a carbon dioxide value of 5.2. Thus the relationship between the alveolar respiratory quotients and the alveolar carbon dioxide is most marked at the extreme limits of the ranges of both values, that is, the higher the alveolar carbon dioxide, the lower the alveolar respiratory quotient. The alveolar respiratory quotients which vary from 0.80 to 0.92 are probably due to the changes in the character of the metabolism and these correspond to the changes in the respiratory quotient of the expired air.

With A. F. S. on June 3, there is more of a relationship between the respiratory quotient of the alveolar air and the carbon dioxide of the alveolar air than in the experiments with J. C. The quotients ranged, in the majority, between 0.75 and 0.91, and the alveolar carbon dioxide ranged between 5.15 and 5.7 per cent. The highest respiratory quotients of 1.02, 0.956, and 0.940 correspond to low carbon dioxide of the alveolar air, namely, 5.00 and 5.08 and 5.03. These extremes are probably abnormal, both with respect to the respiratory quotient and the alveolar air. In other words, one would scarcely be so sure of drawing the conclusion that either

all the alveolar respiratory quotients or the respiratory quotients of the expired air represented metabolic changes. The results with A. F. S. are for the most part similar to those obtained with J. C. but had a wider range in both alveolar carbon dioxide and alveolar respiratory quotient.

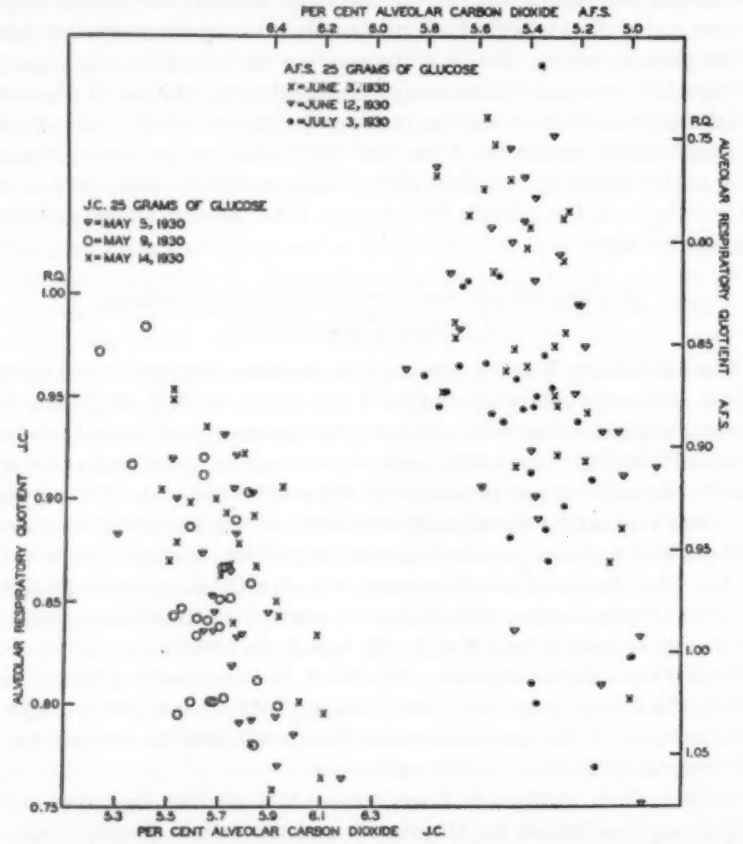

FIG. 9. Diagram showing relationship between alveolar respiratory quotient and percentage alveolar carbon dioxide in experiments with J. C. and A. F. S. before and after ingestion of 25 grams of glucose. The experiments with A. F. S. are plotted in an inverse manner to those of J. C.

The alveolar respiratory quotients as well as the quotients of the expired air are not so significant with A. F. S. as indications of the metabolic changes as are those of J. C. because of the shifts in the percentages of the alveolar carbon dioxide during the course of the observations. To be

of significance the alveolar carbon dioxide should remain practically constant throughout the periods.

A plotting of the relationships between the alveolar respiratory quotient

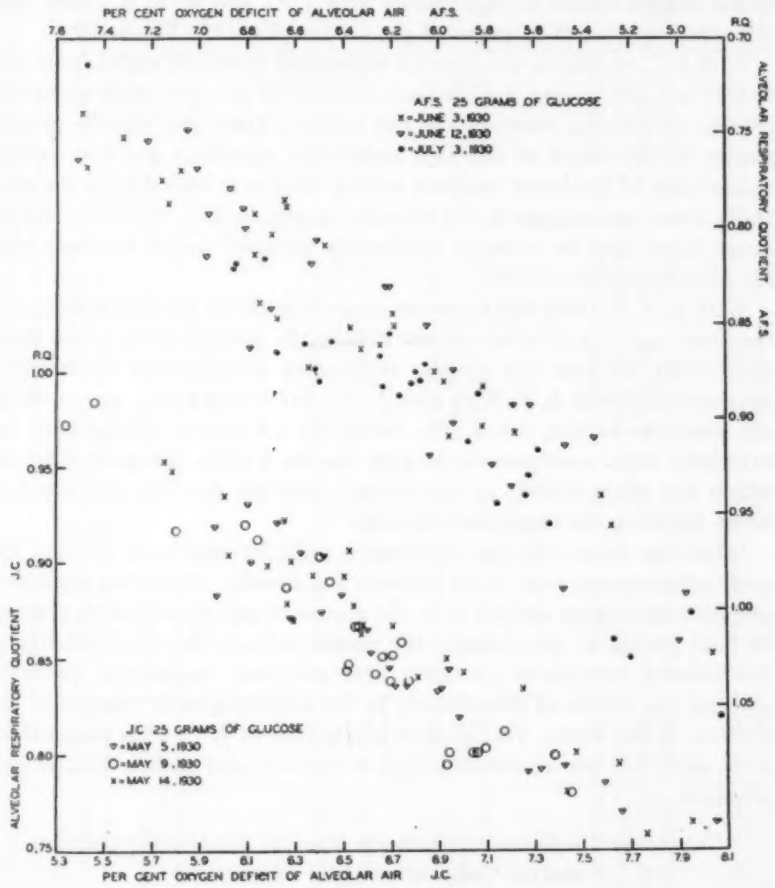

FIG. 10. Diagram showing the relationship between the alveolar respiratory quotient and the alveolar oxygen deficit in experiments with J. C. and A. F. S. before and after the ingestion of 25 grams of glucose. The experiments with A. F. S. are plotted in an inverse manner to those of J. C.

and the alveolar carbon dioxide in the experiments with fructose shows essentially the same results as the experiments with glucose.

Relationships between Alveolar Respiratory Quotients and Percentage Alveolar Oxygen Deficits

The relationships between the alveolar respiratory quotient and the alveolar oxygen deficit in experiments with J. C. and A. F. S. before and after the ingestion of 25 grams of glucose are plotted in Figure 10.

With J. C. on May 5, the alveolar respiratory quotients varied from 0.76 to 0.93 and the oxygen deficits from 6.0 to 8.05 per cent, with a distinct inverse relationship between the two factors. There is a slightly greater scatter in the values at the high respiratory quotients and low oxygen values than in the lower quotient values. This is in part due to the generally lower percentages in the alveolar carbon dioxide. All three experiments show that the alveolar respiratory quotient varied inversely with the alveolar oxygen deficit.

With A. F. S. there was a greater range in both the alveolar respiratory quotients and the alveolar oxygen deficits. In general there is the same relationship between the alveolar respiratory quotient and the alveolar oxygen deficit with A. F. S. as with J. C., that is, the higher the quotient, the lower the oxygen deficit. The extremely low oxygen deficits with the extremely high quotients are in part due to a more dilute alveolar air which was characteristic of this subject after the first one and one-half hours following the ingestion of glucose.

When the values for the experiments with fructose were plotted, the same relationships were found between the alveolar respiratory quotients and alveolar oxygen deficits as in the groups of experiments with glucose. In both groups of experiments, the results indicate that the majority of the alveolar respiratory quotients were metabolic respiratory quotients and not the results of disturbances in the breathing or of changes in the reaction of the blood. This is particularly true of J. C. with whom there were relatively few abnormally high or low alveolar carbon dioxide percentages.

Correlation Coefficients between the Alveolar Respiratory Quotient and the Composition of the Alveolar Air

In Table I are given the arithmetical means of the alveolar respiratory quotient, alveolar carbon dioxide and alveolar oxygen deficit with the average variations from the means and the standard deviations, and the correlation coefficients between the alveolar respiratory quotient and the alveolar carbon dioxide and the alveolar oxygen for J. C. and A. F. S. in the experiments with 25 grams of glucose or 25 grams of fructose.

TABLE I

CORRELATION COEFFICIENTS BETWEEN THE ALVEOLAR RESPIRATORY QUOTIENTS AND THE ALVEOLAR CARBON DIOXIDE AND OXYGEN DEFICIT IN EXPERIMENTS WITH GLUCOSE AND FRUCTOSE

Subject and date	Number of determinations	Alveolar R.Q.		Alveolar CO_2		Alveolar O_2 deficit		Correlation coefficients			
		Average	S.D.	Average	S.D.	Average	S.D.	Alveolar R.Q. and alveolar CO_2		Alveolar R.Q. and alveolar O_2 deficit	
1930				%		%		r	P.E.$_r$	r	P.E.$_r$
25 grams of glucose											
J. C.											
May 5	25	0.850±0.041	0.048	5.74±0.12	0.17	6.75±0.45	0.55	−0.629±0.082		−0.949±0.013	
May 9	26	0.857±0.042	0.053	5.65±0.12	0.16	6.59±0.38	0.51	−0.588±0.087		−0.961±0.010	
May 14	26	0.865±0.041	0.052	5.77±0.15	0.19	6.67±0.48	0.59	−0.700±0.067		−0.964±0.009	
A. F. S.											
June 3	29	0.845±0.052	0.065	5.40±0.17	0.21	6.40±0.52	0.65	−0.621±0.077		−0.956±0.011	
June 12	30	0.856±0.074	0.094	5.34±0.20	0.24	6.30±0.72	0.91	−0.486±0.094		−0.954±0.011	
July 3	30	0.902±0.057	0.076	5.45±0.18	0.25	6.06±0.52	0.65	−0.405±0.103		−0.925±0.018	
25 grams of fructose											
J. C.											
April 21	25	0.862±0.052	0.060	5.89±0.15	0.20	6.84±0.54	0.62	−0.529±0.097		−0.941±0.016	
April 23	24	0.865±0.043	0.058	5.87±0.12	0.16	6.79±0.42	0.54	−0.532±0.099		−0.955±0.012	
April 28	26	0.861±0.054	0.062	5.84±0.10	0.13	6.79±0.46	0.53	−0.258±0.123		−0.960±0.010	
May 12	25	0.905±0.048	0.066	5.97±0.14	0.18	6.61±0.51	0.72	−0.825±0.043		−0.979±0.006	
A. F. S.											
May 23	30	0.793±0.047	0.057	5.75±0.18	0.20	7.26±0.61	0.72	−0.611±0.077		−0.948±0.013	
June 10	30	0.838±0.090	0.106	5.55±0.19	0.23	6.71±0.91	1.04	−0.531±0.088		−0.976±0.006	
July 2	30	0.884±0.092	0.124	5.42±0.27	0.33	6.27±0.96	1.27	−0.623±0.075		−0.956±0.011	

The correlation coefficients between the alveolar respiratory quotient and the alveolar carbon dioxide in the experiments with glucose indicate a tendency for the alveolar carbon dioxide to vary inversely as the alveolar respiratory quotients. This is logical because the alveolar carbon dioxide will depend to a large extent on the correctness of the sampling. If the sample is drawn at the time of over-ventilation the alveolar air will be diluted as well as extra carbon dioxide washed out, and the respiratory quotient will be too high. Conversely, if the alveolar ventilation is inadequate, the alveolar carbon dioxide will be too high and the alveolar respiratory quotient too low.

The correlation between the alveolar respiratory quotient and the alveolar oxygen deficit was high in this group of experiments with both subjects. The alveolar respiratory quotient varied inversely as the oxygen deficit. In spite of the variations in alveolar carbon dioxide, the oxygen deficit varies inversely much more closely with the alveolar respiratory quotient than does the alveolar carbon dioxide. In fact, the correlation coefficients between the alveolar oxygen deficit and the alveolar respiratory quotient are higher than the correlation coefficients between the oxygen deficit of the expired air and the respiratory quotient of the expired air (unpublished). The alveolar air is not directly influenced by either the dead space or the respiration rate, as is the composition of expired air.

The correlation coefficients of the alveolar carbon dioxide and the alveolar respiratory quotient in the experiments with fructose were all negative, thus indicating that the carbon dioxide varied inversely as the quotient. However, only half of them are above 0.6, and the coefficients of the others hardly exceed 6 times the probable error. The correlation between the alveolar carbon dioxide and alveolar respiratory quotient is not significant. The correlation coefficients of the alveolar respiratory quotient and the alveolar oxygen deficit were negative and of a very high order, that is, the respiratory quotient varied inversely as the oxygen deficit of the alveolar air, and this applies without exception to both subjects.

Comparison of Alveolar Air during the Base-line Hour and the Period of Maximum Respiratory Quotient after the Sugars

Figures 1 to 4 show the general course of the respiratory quotients of expired air before and after the ingestion of glucose and of fructose and the course of the percentage of carbon dioxide in the alveolar air, and in Figures 5 to 8 are shown the averages of the alveolar carbon dioxide, the alveolar respiratory quotient, and the respiratory quotient of the expired

air. In some experiments there were marked variations and a closer analysis of the possible changes in the respiratory quotient due to the ingestion of the sugars is desirable. For this purpose Table II has been prepared. In

TABLE II.

COMPARISON OF ALVEOLAR CARBON DIOXIDE AND RESPIRATORY QUOTIENT DURING BASE-LINE HOUR WITH THOSE DURING PERIODS OF MAXIMUM RESPIRATORY QUOTIENT AFTER INGESTION OF 25 GRAMS OF GLUCOSE AND OF FRUCTOSE

Subject and date	Base-line hour			Periods of maximum R.Q. after sugars		
	(a) Average R.Q.	(b) Average alveolar CO_2 %	(c) Average alveolar R.Q.	(d) Average R.Q.	(e) Average alveolar CO_2 %	(f) Average alveolar R.Q.
1930						
25 grams of glucose						
J.C.						
April 30	0.840	5.87	—	0.885	5.85	—
May 5	0.805	5.86	0.805	0.885	5.65	0.895
May 9	0.785	5.71	0.835	0.860	5.63	0.900
May 14	0.830	5.77	0.860	0.895	5.80	0.895
Average	0.815	5.80	0.830	0.880	5.73	0.895
A. F. S.						
June 3	0.770	5.53	0.785	0.845	5.36	0.890
June 12	0.750	5.43	0.815	0.870	5.11	0.935
July 3	0.795	5.72	0.890	0.890	5.25	0.970
Average	0.770	5.56	0.830	0.870	5.24	0.930
25 grams of fructose						
J. C.						
April 21	0.795	5.94	0.820	0.905	5.90	0.910
April 23	0.800	5.96	0.830	0.935	5.81	0.935
April 28	0.810	5.86	0.840	0.925	5.86	0.935
May 12	0.890	5.84	0.900	0.975	5.87	0.970
Average	0.825	5.90	0.850	0.935	5.86	0.940
A. F. S.						
May 23	0.755	6.00	0.740	0.890	5.63	0.885
June 10	0.745	5.61	0.785	0.875	5.61	0.925
July 2	0.755	5.90	0.810	0.960	5.11	1.045
Average	0.750	5.84	0.780	0.910	5.45	0.950

column (a) is given the average base-line respiratory quotient for each experiment which is made up of periods 2 to 4 for the most part. The column (b) shows the average per cent of carbon dioxide in the alveolar air for the same periods. In column (c) is given the average alveolar respiratory quotient for the corresponding periods. In the right hand portion of the table there is given the average [column (d)] of the respiratory quotients of expired air of the three periods which were the highest after ingestion of the sugar. In nearly all cases with glucose these began in the ninth or tenth period. On May 9 the average included the four periods 9 to 12 and on May 14, periods 8 to 10, that is, in practically all cases the maximum respiratory quotient occurred at about the same interval of time after the ingestion of sugar. In the experiments with fructose the highest quotients were in the periods 7 to 9 with J. C. and 8 to 10 with A. F. S. In column (e) is given the average alveolar carbon dioxide for the periods selected for the highest respiratory quotient. In column (f) is given the average alveolar respiratory quotient for these periods. With J. C. the average respiratory quotient in the base-line hour in the experiments with glucose varies from 0.78 to 0.84, but the alveolar carbon dioxide has a range of only 0.16 per cent for the 4 experiments. In two cases the average alveolar respiratory quotient was higher than the average respiratory quotient of the expired air, and in these two cases the average alveolar carbon dioxide was slightly lower than the other two of the four. The difference between the respiratory quotient of expired air and the alveolar respiratory quotient is not large, viz., 0.015 on the average. In two of the experiments the average alveolar carbon dioxide is practically the same in the periods of maximum respiratory quotient as that during the base-line hour. In the other two these percentages are 0.08 and 0.21 lower than those in the base-line hour, and in these two (May 5 and May 9) the average alveolar respiratory quotient after glucose is slightly higher than the average respiratory quotient of the expired air. The average alveolar carbon dioxide percentage after the sugar is lower by 0.07 than the average during the base-line hour. After glucose the difference between the average respiratory quotient of the expired air and the average respiratory quotient of the alveolar air is 0.015.

With A. F. S. the average respiratory quotient in the base-line hour in the experiments with glucose as well as the percentage of carbon dioxide in the alveolar air was materially lower than with J. C. and the difference between the respiratory quotient of the expired air and that of the alveolar air was more marked, being 0.06 on the average. The average value of the respiratory quotient of the periods of maximum height was 0.10 higher

than that of the base-line hour, a greater difference than was obtained with J. C. Similarly, the average percentage of carbon dioxide of the alveolar air for the periods of maximum respiratory quotient was lower by 0.32 than that of the base-line hour. There was also, on the average, a much higher alveolar respiratory quotient during the periods of maximum respiratory quotient than was obtained with J. C., and the difference between the respiratory quotient of the expired air and that of the alveolar air was 0.06. Therefore, with J. C. it is apparent that the average maximum respiratory quotient after the ingestion of glucose is of significance because there was but little change in the percentage of carbon dioxide in the alveolar air, and the respiratory quotients of the alveolar air agreed in the main with those of the expired air. On the contrary, with A. F. S. part of the rises in the respiratory quotient after the ingestion of glucose must have been due to a fall of the alveolar carbon dioxide, so that there were combined the effects of the increase in the metabolism of carbohydrates due to the utilization of glucose and of a fall of the carbon dioxide content of the body, with the result that the respiratory quotient was raised more in the case of A. F. S. than with J. C.

Additional evidence may be obtained from a consideration of the course of these factors for the same periods of time in the group of experiments in which no dose was given (see preceding article). In Table III are grouped for the experiments with no dose the values for the periods of time corresponding to those with the ingestion of glucose, particularly with reference to the maximum respiratory quotient after the ingestion. With J. C. there is a satisfactory agreement in the respiratory quotient for the two groups of periods as well as for the carbon dioxide of the alveolar air. This is true not only with respect to the average, but also with respect to the individual experiments so far as the alveolar air is concerned. There are only two experiments in which the respiratory quotient was determined for the alveolar air, but the differences between the average alveolar respiratory quotient and that of the expired air are not wide. On the contrary, with A. F. S. there is a marked difference between the average respiratory quotient of the base-line hour and that of the periods corresponding to the maximum respiratory quotients in the glucose experiments. There was an increase of 0.04 in the respiratory quotient and a decrease of 0.45 in the alveolar carbon dioxide percentage, thus indicating an approximate increase in the respiratory quotient of 0.01 for each fall in the alveolar carbon dioxide percentage of 0.10. If we apply a correction of this character to the values obtained in Table II after the ingestion of glucose, we shall

TABLE III

AVERAGE ALVEOLAR CARBON DIOXIDE AND RESPIRATORY QUOTIENT FOR THE SAME PERIODS IN NO-DOSE EXPERIMENTS AS THOSE COMPARED FOR THE MAXIMUM RESPIRATORY QUOTIENTS IN THE GLUCOSE AND FRUCTOSE EXPERIMENTS

Subject and date	Preliminary hour			No-dose periods*			No-dose periods**		
	(a) R.Q.	(b) Alveolar CO_2 %	(c) Alveolar R.Q.	(d) R.Q.	(e) Alveolar CO_2 %	(f) Alveolar R.Q.	(g) R.Q.	(h) Alveolar CO_2 %	(i) Alveolar R.Q.
1930									
J.C.									
April 14	0.795	5.92	—	0.795	—	—	0.780	—	—
April 16	0.825	6.08	—	0.800	5.92	—	0.805	5.92	—
April 18	0.830	6.30	—	0.795	6.22	—	0.800	6.24	—
May 7	0.765	5.96	0.790	0.790	6.09	0.765	0.790	6.01	0.785
May 16	0.820	5.86	0.830	0.800	6.02	0.785	0.810	5.89	0.825
Average	0.805	6.02	0.810	0.795	6.06	0.775	0.800	6.02	0.805
A. F. S.									
May 27	0.770	5.73	0.800	0.775	5.68	0.785	0.775	5.73	0.795
June 26	0.810	5.98	0.860	0.860	5.42	0.895	0.850	5.51	0.895
June 28	0.775	6.07	0.800	0.835	5.33	0.880	0.830	5.46	0.855
Average	0.785	5.93	0.820	0.825	5.48	0.855	0.820	5.57	0.850

* Corresponding to periods of highest respiratory quotients in the experiments with glucose.

** Corresponding to periods of highest respiratory quotients in the experiments with fructose.

have a decrease of the average respiratory quotient after the ingestion of glucose of 0.03 corresponding to a difference of 0.32 between the average alveolar carbon dioxide in the base-line hour and that in the periods of maximum respiratory quotient. This decreases the difference between the average respiratory quotient of the expired air during the base-line hour and the periods of maximum respiratory quotient to 0.070, which is practically the same as the difference between the averages found with J. C. Therefore, when the respiratory quotient of the maximum periods is corrected for the possible change due to a fall of alveolar air based upon experiments in which no sugar was given, we find that the rise in respiratory quotient due to glucose alone is practically the same in both cases. This method of analysis of the results with A. F. S. makes useful experiments on him which would otherwise be of doubtful significance. The results with A. F. S. (an untrained subject) thus supplement and confirm the results found with J. C., a trained subject.

In the experiments with fructose (Table II) the averages of the alveolar CO_2 for the base-line hour with J. C. show a narrow range, 0.12 per cent, from day to day, with an average of 5.90 for the four days. The average respiratory quotient of the alveolar air was slightly higher than that of the expired air.

The average maximum respiratory quotient of J. C. after the ingestion of fructose was 0.935, and this rise of 0.11 over the base-line hour occurred in the periods 7 to 9 after the ingestion of fructose. The differences in three of the four experiments were close to the average difference. The average carbon dioxide of the alveolar air in the periods of maximum R. Q. was remarkably close to that of the average alveolar carbon dioxide in the base-line hour, both with regard to the individual experiments and the averages of the four. The agreement indicates that the increase in respiratory quotient was a metabolic one, that is, due to the transformation of carbohydrates. The average respiratory quotient of the alveolar air was also close to that of the expired air. Thus with J. C. the evidence is very consistent with regard to the influence of the ingestion of fructose on the respiratory exchange in indicating that the rise in the respiratory quotient was due to the metabolic changes which took place after the ingestion of fructose.

With A. F. S., the average respiratory quotient in the base-line hour was lower than with J. C., although the alveolar carbon dioxide of A. F. S. was very close to that of J. C. There is not a marked difference between the average respiratory quotient of the alveolar air and that of the expired air during the base-line hour. The difference between the respiratory quotient

of the base-line hour and the maximum respiratory quotient after fructose was 0.160, which is a larger increase than that of J. C. The average alveolar carbon dioxide in two of the three experiments was lower than that of the base-line hour and the average respiratory quotient of the alveolar air was somewhat higher than that of the expired air.

In Table III are given the average respiratory quotients for the periods in the post-absorptive experiments given in a preceding article (see p. 37) corresponding to those of the maximum respiratory quotients after the ingestion of fructose. With J. C. there is no difference between the base-line hour and the periods of maximum respiratory quotient, in the respiratory quotient of expired air, carbon dioxide of alveolar air, and alveolar respiratory quotient. With A. F. S., on the contrary, there is a higher respiratory quotient in the periods corresponding to the maximum with fructose and also a lower alveolar carbon dioxide per cent as well as a high alveolar respiratory quotient. If we correct the increase in respiratory quotient after the ingestion of fructose with A. F. S. by 0.01 corresponding to each 0.10 per cent decrease in the alveolar carbon dioxide, we shall have the same increase in the respiratory quotient of the expired air with A. F. S. as with J. C., namely, 0.12 with A. F. S. as compared with 0.11 with J. C. Therefore the greater portion of the rise in the respiratory quotient with A. F. S. was due to the metabolic process after the ingestion of fructose and but a small proportion due to the fall of the alveolar carbon dioxide. The maximum respiratory quotient after the ingestion of 25 grams of fructose is 0.05 greater than the maximum rise in the respiratory quotient after the ingestion of glucose and there is, therefore, a marked difference between the effects of fructose and glucose upon the respiratory exchange. Although the difference in the maximum rise in the quotient may not appear to be large enough to be considered of much significance, it should be noted that the quantities of sugar ingested were small (only 25 grams) in comparison with the amounts used with human subjects, and the differences are always found consistently when these two sugars are used.

Summary

The respiratory exchange and composition of the alveolar air were determined with two human subjects on 3 and 4 days for four post-absorptive 15-minute periods and for ten 15-minute periods after the ingestion of 25 grams of glucose and of fructose.

There was no change in the alveolar carbon dioxide with the trained subject accompanying the rises in the respiratory quotient after the inges-

tion of the sugars, but with an untrained subject there was a marked fall in the alveolar carbon dioxide during the periods of maximum rise in the respiratory quotient.

With the trained subject there was not a definite relationship between the alveolar carbon dioxide and the alveolar respiratory quotient in the experiments with fructose, but in the experiments with glucose and in the two groups of experiments with the untrained subject there was a tendency to a negative correlation between the alveolar carbon dioxide and the alveolar respiratory quotient. There was a marked negative correlation between the alveolar oxygen deficit and the alveolar respiratory quotient in both groups of experiments with both subjects. The alveolar respiratory quotients tended to run parallel with the respiratory quotients of the expired air.

There was a maximum rise in the respiratory quotient of 0.065 in the experiments with glucose with one subject and 0.10 with the other subject. When the respiratory quotient after the ingestion of glucose with the untrained subject was corrected for the rise in respiratory quotient corresponding to the falls in the alveolar carbon dioxide equivalent to those which took place after the ingestion of glucose, the net increase in the respiratory quotient was then the same as with the trained subject.

The average maximum rise in the respiratory quotient of the expired air with the trained subject in the experiments with fructose was 0.11 and was accompanied by nearly the same rise in the alveolar respiratory quotient with no significant change in the alveolar carbon dioxide. The average maximum rise in the respiratory quotient of the expired air with the untrained subject was 0.16, accompanied by a rise of 0.17 in the alveolar respiratory quotient and a decrease in the alveolar carbon dioxide of 0.4 per cent. When the respiratory quotient of the expired air is corrected for the apparent rise due to a fall of alveolar carbon dioxide found in previous post-absorptive experiments with this subject, the increase in the respiratory quotient of the expired air becomes 0.12, thus practically the same as with the trained subject.

Therefore a comparison of the course of the alveolar air and the respiratory quotient in the post-absorptive condition with the changes taking place after the ingestion of sugars gives a basis for the calculation of the true effect of the ingested sugars on the respiratory exchange.

If the constancy of the alveolar air is to be taken as an indication of true metabolic respiratory quotients, as has been frequently suggested in the literature, then the net rise in respiratory quotients in these experiments

as the result of ingestion of fructose must be regarded as a result of the metabolism of fructose without the formation of organic acids in its transformation in sufficient quantities to affect the carbon dioxide tension of the alveolar air.

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A COMPARISON OF APRICOTS AND THEIR CAROTENE EQUIVALENT AS SOURCES OF VITAMIN A

By

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THE recent experimental work on vitamin A and carotene points definitely to a correlation between these two substances. While the evidence is against their being identical, there is ample reason to assume that carotene is a source of vitamin A and may perhaps be considered its pro-vitamin. The work to date shows that the pro-vitamin A is synthesized by plants and is found in the highest concentrations in the green and yellow leaves, seeds and roots. The flowers, fruits, and vegetables which have been shown to contain an abundance of pro-vitamin A are rich in the carotenoid pigments and in many cases chlorophyll is also present. In any case, carotene appears to be closely associated with the formation of vitamin A or a pro-vitamin A in plants and with its utilization by animals. The question of the quantitative equivalence of the two substances cannot be said to have been established so far. It seemed worth while then to attack this question by simultaneous chemical and biological analyses of a typical food containing carotene and acting as source of vitamin A in measurable amounts. The fruit chosen was the apricot, partly because its vitamin A value had already been studied rather exhaustively in this laboratory and partly because of its exceptional activity as source of vitamin A and its concurrent richness in carotenoid pigments.

The plan of experiments here reported involved:

1. The quantitative estimation of the carotene contents of the apricot samples used, viz. fresh, frozen, unsulfured sun-dried, and sulfured sun-dried specimens.

2. The biological testing of these same samples for vitamin A activity.

3. The biological testing of the vitamin A value of crude and purified carotene fed in amounts equivalent to some of those found in the apricot samples and used in the vitamin A testing of the fruit.

There should emerge from these determinations some suggestions as to the truth of the supposition of the dependence of the vitamin value of the fruit samples upon their carotene content. Likewise, the interrelation of

amount of destruction of both carotene and vitamin A simultaneously in the dried samples of the apricots might offer additional evidence as to the equivalence of these two substances.

Royal apricots, having a deep yellow flesh, were chosen for the test, and the same variety lot, and crop were used in the three forms tested. All the fruit samples were taken from the same orchard. The fresh fruit was preserved by freezing and storage at $-17°$ after having been packed in small cans which were then evacuated and refilled with nitrogen twice. Two sun-dried samples were used, one of which was sulfured for 4 hours. The fresh fruit, S1, contained 79.4 per cent moisture, the sulfured sun-dried sample, S2, had a moisture content of 17.5 and contained 480 parts of sulfur dioxide per million. The unsulfured sun-dried sample, S3, contained 14.7 per cent water.[1]

The carotene contents of the fruits were colorimetrically determined on the petroleum ether and ether soluble fractions. The usual biological test with rats was used for vitamin A efficiency of the fruits and of the carotene which was obtained from the British Drug Houses, Ltd. and purified by recrystallization from petroleum ether.

The carotene of the samples was extracted by two methods, by pyridine as suggested by Smith and Spoehr (1) and developed by L. L. W. Smith in this laboratory (2) and by acetone and ether as described by Schertz (3). The pyridine was used in several portions, about 15 times the weight of the sample (which was usually 10 grams) being needed for complete extraction of the carotenoid pigments. The pyridine was removed with normal sulfuric acid and the pigment taken up in petroleum ether, dried with anhydrous sodium sulfate, made up to volume and the color read against the carotene standard of Sprague (4).

The acetone-ether extraction was carried out as modified by Schertz (3). The acetone and flavones were washed out with water and finally with one per cent sodium carbonate solution. The ethyl ether extract was evaporated at a temperature below 50° in a current of carbon dioxide. The residue was shaken up with petroleum ether and methyl alcohol, the latter taking up any xanthophyll which was present. But in the case of apricots xanthophyll appears to be absent since the methyl alcohol washings were colorless. The petroleum ether layer was then washed with water, dried with anhydrous sodium sulfate, made up to volume and read against the Sprague standard.

[1] The fruit was prepared and this analysis furnished by P. F. Nichols of the Fruit Products Division, College of Agriculture, University of California.

TABLE I

COMPARATIVE EXTRACTIONS OF CAROTENE BY PYRIDINE AND ACETONE-ETHER

Sample	Number of determinations	Carotene per gram of fruit				Carotene per gm. of fruit solids
		Acetone-ether extractions	Pyridine extract of acetone-ether extracted residues	Acetone-ether extract and pyridine extract of residues	Pyridine extractions	
		mg.	mg.	mg.	mg.	mg.
Sl. Fresh frozen apricots, 1930 crop	4	—	—	—	0.021	0.102
	5	0.018	—	—	—	0.088
Fresh apricots, 1931 crop	5	0.023	—	—	—	—
	3	—	—	—	0.024	0.115
S2. Sulfured sun-dried apricots	4	0.041	0.014	0.056	—	0.066
	3	—	—	—	0.055	0.066
	4	0.042	—	—	—	0.050
S3. Unsulfured, sun-dried apricots	4	—	—	—	0.051	0.060
	4	0.040	—	—	—	0.047
	6	0.039	0.011	0.050	—	0.059

The pigment values found by these 2 methods as shown in Table I do not agree closely, the pyridine extracts being in all cases except that of the fresh fruit richer in carotene than the acetone-ether extracts. It was at first suspected that some loss occurred during the evaporation of the ethyl ether extract in the latter process. Since xanthophylls appear to be negligible in amount in apricots, the methyl alcohol separation and ether evaporation were omitted in a new trial of the Schertz separation and values were obtained identical with those found when these steps were not omitted. The pulp residues from the acetone-ether extractions were then further extracted with pyridine by the method described and when the resulting solutions were read colorimetrically against the standard were found to contain enough pigment to account for the difference between the original pyridine and acetone-ether extracts. It is thus evident that pyridine removes pigments, particularly from the dried fruit samples, which the acetone and ether had not extracted. The nature of this pigment is doubtful. It may be either carotene or lycopersicin since Smith and

Smith (5) found the latter pigment to be more readily soluble in pyridine than in acetone and ether.

The fresh frozen apricots which were used for the vitamin tests were found to have a maximum of 0.102 mg. carotene per gram of fruit solids as compared with 0.066 and 0.060 for the sulfured and unsulfured samples, indicating losses of 36 and 41 per cent of the carotenoid values during the drying process. Interest attaches to the corresponding losses in vitamin A potency of these dried fruits.

TABLE II

THE VITAMIN A ACTIVITY OF FRESH AND DRIED APRICOTS

Sample	Daily dose		Number of rats	Body weights		Total gain	Period	Weekly gains
		Equivalent in fresh fruit		Initial	Final			
	mg.	mg.		gms.	gms.	gms.	days	gms.
Fresh frozen apricots, S1	0.0	0	21	79	75		20	−1.3
	15.0	15	12	68	83	15±3	35	3.0
	25.0	25	10	75	105	30±3	46	4.6
	30.0	30	19	62	101	39±1	52	5.2
	40.0	40	7	54	108	54±5	54	7.8
	50.0	50	5	71	140	69±4	56	8.6
Sulfured sun-dried apricots, S2	7.5	30	10	66	86	20±2	38	3.6
	22.5	90	13	80	129	49±3	54	6.3
	30.0	120	16	103	154	51±2	53	6.7
	37.5	150	9	75	138	63±2	56	7.9
Unsulfured sun-dried apricots, S3	7.5	31	12	68	89	21±2	40	3.6
	22.5	93	16	109	137	28±3	49	4.0
	30.0	124	20	106	148	42±2	54	5.4

The vitamin tests were made by the usual biological method, both loss of weight and appearance of ophthalmia being used as criteria of vitamin A depletion. The doses of apricot used were chosen in accord with previous findings (6) in this laboratory on similar samples. As shown in Table II, the 15 mg. daily dose of the fresh fruit supports an increase of 3 grams body weight per week, the usual Sherman standard for the vitamin A unit. Twice the equivalent of this amount must be fed as either sulfured or unsulfured sun-dried apricot in order to obtain about the same amount of growth. A similar relation appears to be shown by the effects of the larger doses, 25 and 30 mg. of the fresh fruit giving about the same rate of in-

crease as 90 to 120 mg. (fresh equivalent) of the sulfured dried fruit or 93 to 124 mg. of the unsulfured fruit. It is fair to say then that only 25 to 50 per cent of the vitamin A activity of the fresh fruit is retained in the dried specimens. The loss of vitamin cannot be stated so exactly as can the corresponding loss of carotene content, but it is apparent that these losses are of the same general magnitude, although the carotene destruction tends to be distinctly lower, 36 to 41 per cent as compared with 50 to 75 per cent loss of vitamin, possibly because of better absorption of carotene from the fresh fruit.

Crystalline carotene of melting point 162°–164° was obtained from the British Drug Houses, Ltd. and was fed in ethyl laurate[2] in doses of 0.001, 0.002, and 0.005 mg. daily. Excellent growth was obtained on the larger doses and at the rate of 4.3 grams per week even on the 0.001 mg. dosage. The carotene was then recrystallized from its filtered solution in boiling purified petroleum ether, (boiling point 30°–60°) three times with rise in melting point to 179°–180°. A fourth recrystallization of part of the material raised the melting point to 181°. The crystals which corresponded closely with the description of the crystals obtained by Olcovich and Mattill (7) were dried in an atmosphere of carbon dioxide and kept in the dark. The ethyl laurate solution used for feeding was made up, in small, frequently renewed portions, from the third crystal crop. Doses of 0.001, 0.002 and 0.005 mg. daily of the purified carotene as indicated in Table III

TABLE III

VITAMIN A ACTIVITY OF CAROTENE

Description of sample	Daily dose	Number of rats	Body weights		Total gain	Period	Weekly gains
			Initial	Final			
	mg.		gms.	gms.	gms.	days	gms.
Crude carotene, m.pt. 162°–164°	0.001	10	80	112	32±7	52	4.3
	0.002	10	79	135	56±6	53	7.4
	0.005	6	91	163	72±4	56	9.0
Crystalline carotene, m.pt. 179°–180°	0.001	4	118	171	53±5	54	6.9
	0.002	4	111	198	87±6	56	10.9
	0.005	4	105	209	104±4	56	13.0

produced distinctly better growth in vitamin A depleted rats than did the similar doses of the crude carotene. The vitamin activity appeared to be

[2] The ethyl laurate was kindly supplied by S. Lepkovsky of the Anatomical Laboratory of the University of California.

considerably increased by the purification, an outcome not unexpected if the crude substance contained oxidized or otherwise changed carotene products which were removed by the recrystallization. It is possible also that the original carotene contained a larger proportion of the less active alpha-carotene than that recrystallized from petroleum ether solution. The melting point of the alpha isomer is usually given as several degrees lower than that of the beta form. Kuhn and Lederer (8) found the solubility in n-hexane of the alpha isomer to be three times that of the beta-carotene and Kuhn and Brockman (9) as well as Karrer, Euler, Hellstrom, and Rydbom (10) report a slightly greater and more rapid response from beta than from alpha-carotene when used as source of vitamin A.

The growth of 4 rats at an average rate of 6.9 grams per week on 0.001 mg. carotene daily, about the same rate produced by twice this dosage of the crude carotene, represents perhaps the best results so far reported with carotene as source of vitamin A. Collison, Hume, Smedley-Maclean, and Smith (11) and Moore (12) were able to get some cures of vitamin A deficient animals with 0.002 mg. carotene but Green and Mellanby (13) found the minimum dose, melting point 174°, necessary for complete protection to be somewhat larger, 0.020 mg. Olcovich and Mattill (7) used 0.005 mg. as their lowest dose. Kuhn and Brockmann (9) failed to get growth in vitamin A deficient rats with doses of 0.0017 mg. of beta-carotene.

Our rats fed 0.005 and 0.002 mg. of the carotene, melting point 180°, were cured of their deficiency symptoms and all survived the experimental period of 56 days. Three out of four of those given 0.001 mg. survived the full period and grew satisfactorily but on autopsy were found to exhibit internal stigmata of vitamin A deficiency. These stigmata were chiefly crater-like tumors of the stomach and pus infections at the base of the tongue and in the ears and lungs.

The suggestion of Olcovich and Mattill (7) that total growth of rats induced by the administration of a given amount of carotene, as 0.005 mg., be used as criterion of comparative vitamin A value was considered in evaluating our biological tests for carotene. As seen in Table IV however the growth per 0.001 mg. carotene decreases as the total amount fed is increased above the minimum. Thus 0.001 mg. daily fed for 54 days produces weight increases of 0.98 grams per 0.001 mg. carotene but when twice or five times that amount is given the figure drops to 0.78 and 0.37. This is possibly due to the fact that as Sherman and Burtis (14) have stated, growth beyond the minimum of 3 grams weekly may be limited by other

TABLE IV

RELATION OF CAROTENE INTAKE TO WEIGHT GAINS

Source of of Carotene	Daily dose of substance	Daily dose of carotene	Number of rats	Total amount of carotene fed	Total weight gain	Feeding period	Gain per 0.001 mg. carotene
	mg.	mg.		mg.	gms.	days	gms.
Fresh apricot	15.0	0.00030	12	0.010	15	35	1.50
	25.0	0.00052	10	0.024	30	46	1.24
	30.0	0.00063	19	0.033	39	52	1.18
	40.0	0.00084	7	0.045	54	54	1.20
	50.0	0.00105	5	0.059	69	56	1.17
Sulfured dried apricot	7.5	0.00041	10	0.015	20	38	1.33
	22.5	0.00123	13	0.066	49	54	0.74
	30.0	0.00165	17	0.087	50	53	0.57
	37.5	0.00206	9	0.115	63	56	0.55
Unsulfured dried apricot	7.5	0.00038	12	0.016	21	40	1.31
	22.5	0.00114	16	0.056	28	49	0.50
	30.0	0.00153	20	0.082	42	54	0.51
Crude carotene, m.pt., 162°–164°	0.001	0.001	10	0.052	32	52	0.61
	0.002	0.002	10	0.106	56	53	0.53
	0.005	0.005	6	0.280	72	56	0.26
Recrystallized carotene, m.pt., 179°–180°	0.001	0.001	4	0.054	53	54	0.98
	0.002	0.002	4	0.112	87	56	0.78
	0.005	0.005	4	0.280	104	56	0.37

factors besides vitamin A. In the lower ranges of dosage our figures correspond however very well with those found by Olcovich and Mattill. In doses of 0.0003 to 0.0008 mg. daily the maximum gain of 1.18 to 1.50 gms. per 0.001 mg. carotene was obtained on all our fruit samples; at 0.001 to 0.002 mg. daily the figures drop to 0.50 to 1.17 and at the 0.005 mg. level the growth is only 0.26 and 0.37 gms. per 0.001 mg. carotene. These 3 levels correspond roughly with intakes which produce weekly gains of 3 to 5 gms., 6 to 8 gms. and 9 to 13 gms. for the 8 weeks period.

In nearly all cases the unsulfured dried apricot yielded growth in proportion to carotene intake which was distinctly lower than the other sources of carotene used. This is in accord with our suspicion that certain colored derivatives of carotene or a similar compound present in the unsulfured samples are extracted by both methods used for carotene de-

termination and that our reported figures for the carotene content of these samples are consequently higher than they should be.

Such a comparison of the growth obtained on graded doses of a food in terms of its carotene content may have value in improving the assay for vitamin A. Further standardization of growth responses to varying carotene intakes is needed however.

The responses obtained at approximately the 0.001 mg. level have some interest in connection with the tentative adoption of this dosage of standard carotene as the international unit of vitamin A activity by the Permanent Commission on Biological Standardization of the League of Nations Health Organization (15). The growth response from this amount of carotene or from equivalent amounts of foods should probably be accepted as maximum only when administered at a given daily level and for a definite period. The rate of gain obtained by daily administration of 0.001 mg. carotene in the series here reported, 6.9 mg. per week, is greater than that recommended for the assay of vitamin A activity of foods by Sherman and Munsell (16) but quite close to that suggested by Javillier, Baude, and Levy-Lageunnesse (17), Dutcher, Honeywell and, Dahle (18), and used by Morgan and Field (6).

Thus 0.001 mg. carotene, melting point 180°, produced approximately the same response as did 0.002 mg. carotene, melting point 162°–164°, 40 to 50 mg. fresh apricots, or 22.5 to 30 mg. sulfured dried apricots. The comparative rates of growth secured by the three doses of crystalline carotene and by the most nearly equivalent carotene-containing apricot doses are shown in Chart 1. This dosage of the fresh fruit contained 0.0008 to 0.0010 mg. carotene according to the pyridine extraction figure (Table I) and the sulfured dried fruit 0.0012 to 0.0016 mg. carotene. The largest amount of unsulfured dried apricots, 30 mg., tried did not yield as much curative effect as did 0.001 mg. carotene, although the effect of the smaller doses was comparable with that of like amounts of the sulfured product.

The biological activity of the recrystallized carotene may thus be said to be approximately 25 to 50 per cent greater than that of the crude product. The yield of the purified crystals was considerably less than one-half the weight of the crude product and, as stated before, the proportion of the alpha and beta isomers present in the two preparations may well have been quite different. The biological value of the fresh and sulfured sun-dried apricots may be considered to be approximately 100 per cent of the expected value based on carotene determinations in these fruit samples. The carotene of apricots would seem therefore to be as nearly completely

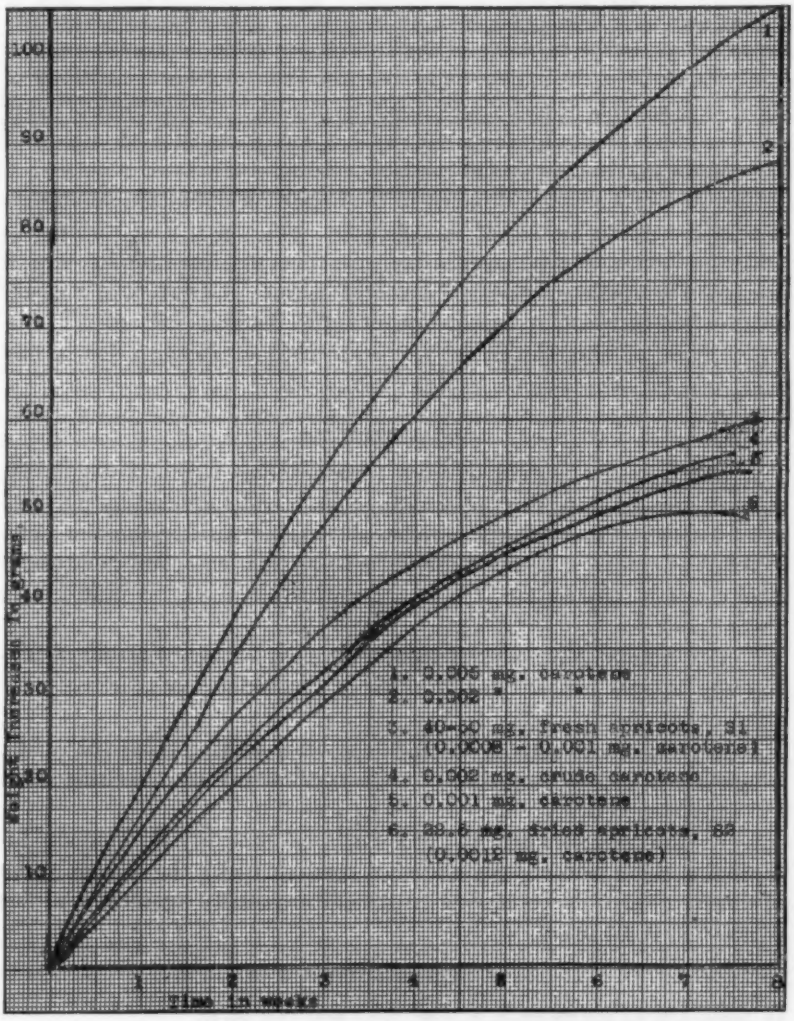

CHART 1. Growth curves of rats fed recrystallized and pure carotene and apricots as source of vitamin A.

convertible to vitamin A by the rat as the present methods of biological testing can determine. This may be tentatively interpreted to mean not only that all of the carotene of the fruit is available for transformation into vitamin A in the animal body, but also that no other constituent of the fruit is capable of such transformation. Whether the carotene of other plant products is capable of yielding such a large proportion of vitamin A can only be determined by further experiments.

The work of Ahmad (19) points to the importance of intestinal absorption in the determination of the efficiency with which food carotene is utilized as source of vitamin A in the animal body. Since completeness of carotene absorption may depend upon the amount and nature of such accompanying materials as fats, the quantitative determination of the carotene content of foods can not as yet be accepted as offering a dependable substitute for the biological testing of the food.

Summary

1. The carotene content of fresh frozen, sulfured sun-dried and unsulfured sun-dried apricots of similar origin was determined by pyridine and by acetone-ether extraction followed by colorimetric estimation. The pyridine was found to extract the dried samples more completely and easily than did the acetone-ether. The loss of carotene in the dried fruit was 36 and 41 per cent.

2. The vitamin A activity of these fruit samples was determined biologically and the loss of vitamin in the dried specimens was found to be 50 to 75 per cent.

3. The vitamin A activity of crude carotene, melting point 162°–164°, was found to be less than that of the crystalline carotene, melting point 180°, made from it.

4. The apricots were seen to yield vitamin A in nearly the amounts indicated by their carotene content when compared with the vitamin value of similar doses of the crystalline carotene. The possible significance of this correlation is discussed.

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THE COPPER, IRON, AND MANGANESE CONTENT OF FISH

By

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THE nutritional value of copper and manganese has recently attracted the attention of investigators. Hart and co-workers (1) were first to show the supplementing effect of copper to iron in hemoglobin formation. Later Titus, Cave, and Hughes (2), Goerner (3), and Myers and Beard (4) found manganese to possess a similar iron-supplementing property. Although the unique rôle of manganese in blood regeneration was not confirmed by Waddell and co-workers (5), Krause (6), Keil and Nelson (7), and Orent and McCollum (8), the experimental evidence, obtained by Mitchell and Miller (9), and Kemmer, Elvehjem, and Hart (10) indicates that manganese stimulates growth, and according to Orent and McCollum (8) manganese is essential for lactation and for the prevention of sterility.

The bearing of these investigations on practical nutrition has aroused an interest in the iron, copper, and manganese content of foods. Although valuable information as to the quantities of these elements contained in a wide variety of foods has been secured by Lindow, Elvehjem, and Peterson (11), Peterson and Elvehjem (12), Lindow and Peterson (13), Remington and Shiver (14), and Orent and McCollum (8), additional data on certain foods are still desirable. The purpose of this paper, therefore, is to report our results on the content of copper, iron, and manganese in twenty species of fish.

EXPERIMENTAL

Preparation of samples. The fish used were bought in the local market and some were caught in Lake Champlain. Only fresh fish were used. After carefully skinning the fish, the edible muscle was stripped from the bones, placed in a prepared glazed porcelain evaporating dish and dried to constant weight at 100° in an electric oven.

Since Remington and Shiver (14), and others, have pointed out that there may be a variation between individuals within the same species, an effort was made to prepare representative samples by finely grinding

and thoroughly mixing together the dried edible muscles from a number of fish of the same kind. The dry, powdered material was finally stored in glass stoppered bottles until the analyses could be made.

Methods of analysis. The copper analyses were made by the method of Elvehjem and Lindow (15). Iron determinations were made by the method according to Stugart (16). Manganese determinations were made by the Skinner and Peterson (17) procedure.

Results

In Table I are given the percentage of moisture and the copper, iron, and manganese content, in mg. per kilo. of fresh moist material, for twenty species of fish.

Table I

Copper, Iron, and Manganese Content of Fish Muscle

Name of Fish	Number used for sample	Moisture	Copper content of fresh muscle	Iron content of fresh muscle	Manganese content of fresh muscle
		per cent	mg. per kg.	mg. per kg.	mg. per kg.
Bass	3	77.0	2.1	4.2	0.26
Bullpout	10	80.0	2.4	9.4	0.24
Cod	5	79.3	3.8	3.4	0.13
Eel	4	55.5	1.7	5.1	0.27
Flounder	5	80.1	1.8	7.0	0.18
Haddock	4	79.1	2.3	4.8	0.15
Halibut	5	78.6	1.8	9.5	0.10
Herring	5	78.2	2.8	5.7	0.16
Herring (fresh water)	6	79.0	3.6	4.8	0.22
Ling	5	78.4	4.1	9.6	0.33
Mackerel	5	76.4	2.6	8.7	0.16
Muskellunge	2	78.8	2.5	6.2	0.23
Perch (yellow)	10	80.4	2.6	5.6	0.44
Pickerel	5	79.7	2.8	6.8	0.18
Pike	5	80.5	1.6	4.5	0.16
Salmon	4	75.0	2.0	8.6	0.14
Shad	5	73.6	2.2	4.8	0.18
Smelt (fresh water)	20	79.5	3.3	4.1	0.26
Sunfish (common)	15	80.3	1.4	3.4	0.29
Trout (lake)	4	78.0	3.1	7.2	0.31

Copper. The average copper content of both the salt water and fresh water fish was about the same. There were approximately 2.5 mg. per kilo.

fresh material in each case. This relationship is similar to that obtained by Lindow and Elvehjem and Peterson (11). They found that the average copper content for sixteen kinds of fish was 2.7 mg. per kilo. of fresh material. We found that the sunfish showed the lowest average copper content while ling was the highest, being 1.4 and 4.1 mg. per kilo. of fresh material respectively.

Iron. Our results in regard to the iron content of salt water and fresh water fish show that the former contain approximately 12 per cent more iron. The results of Peterson and Elvehjem (12) indicated that the salt water fish contained about 40 per cent more iron than was contained in the fresh water fish. This variation in our results is no doubt due to the fact that we analyzed more fresh water fish with dark-colored tissue. Our results are in agreement with those of Peterson and Elvehjem (12) in that the fish with dark colored tissue contained about 75 per cent more iron than those having light colored tissue. This was true in the case of both salt water and fresh water fish. Cod and sunfish contained the lowest amount of iron per kilo. of moist material, being 3.4 mg. in each case. Ling contained the greatest amount, the average amount being 9.6 mg. per kilo. of moist material.

Manganese. The manganese content was slightly greater in the fresh water fish than in the salt water type investigated. The various species of fish muscle contain about 0.1 mg. to 0.4 mg. per kilo. of moist material. This result is in agreement with the observations of Orent and McCollum (8), who state that fish muscle contains 0.1 mg. to 0.5 mg. of manganese per kilo. of moist material. Lindow and Peterson (13) found the muscle of certain species of fish to be manganese free. Skinner and Peterson (17) found that cod contained 6.3 mg. manganese per kilo. of dry material.

Summary

The copper, iron, and manganese content of twenty species of fresh water and salt water fish have been determined. The content of copper in both the fresh and salt water fish averaged about 2.5 mg. per kilo. of moist material. Salt water fish contain about 12 per cent more iron than fresh water fish. Species of fish with dark colored tissue contain approximately 75 per cent more iron than the species with light colored tissue. Fish muscle contains small amounts of manganese.

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THE VITAMIN A CONTENT OF YELLOW-TISSUED AND WHITE-TISSUED APPLES*

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THE nutritional value of apples has not been investigated so extensively as that of some other fruits, although from the standpoint of production the apple stands toward the top of the list. Statistics (1) on the five-year-average car lot shipments of domestic fruits for 1925–26 to 1929–30 show that the car lot shipment of apples exceeds not only that of every other individual fruit, but exceeds as well the combined shipments of all citrus fruits.

Studies on apples and other plant foods have shown that the variety may be a significant factor in the establishment of vitamin values. Bracewell, Hoyle, and Zilva (2) found that the Bramley Seedling variety of apple was more potent in vitamin C than other varieties which they tested. Other studies of Bracewell *et al.* (3) on apple varieties showed that measureable differences existed among varieties in their vitamin C potency. Recent studies of Potter and Dickson (4) on the vitamin A value of different varieties of cherries indicate that certain varieties are more potent in this factor than others. Russell (5) found yellow dent corn to be 50 per cent more potent in vitamin A than a white-capped yellow dent variety. Steenbock and Boutwell (6) found yellow varieties of maize to be far superior to the white varieties as a source of vitamin A. Quinn and Cook (7) in quantitative studies of the vitamin A content of white and yellow yautia, showed the latter to be 10 times as rich in vitamin A as the white variety. Russell (5) has reviewed the more recent investigations concerning the correlation between yellow pigment and the presence of vitamin A.

In this experiment three varieties of apples were studied, two having yellow tissues and one having white tissues. Since yellow pigment has been associated with the presence of vitamin A in other products, it was thought of interest to investigate apples from this standpoint.

Materials and Methods

Thirty albino rats from six litters averaging approximately 41 gm. each in weight were used for the preliminary study. The rats were from stock fed

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the Sherman diet 13 consisting of 2/3 whole wheat, 1/3 whole milk powder, and 2 per cent sodium chloride based on the weight of wheat. The technic of Sherman and Munsell (8) for vitamin A studies was used essentially, and at 21 to 28 days of age the animals were placed on the experiment.

The vitamin A-free basal diet consisted of the following:

	Per cent
Cornstarch, irradiated	67
Casein, vitamin A-free	18
Powdered dry yeast	10
Osborne and Mendel salt mixture	4
Sodium chloride	1
	100

In the preliminary studies, 1930–31, the vitamin D was introduced into the ration by irradiation of the cornstarch with a Cooper-Hewitt Mercury Vapor Quartz Lamp at a distance of 28 inches for a fifteen-minute period. During the 1931–32 experiments, vitamin D was supplied to the animals as 250 D viosterol in the amount of 3 drops weekly per rat. This latter source of vitamin D proved more satisfactory as a few animals gave indications of rib beading when vitamin D was supplied through irradiation of the cornstarch.

The three varieties of apples that were tested for vitamin A were Golden Delicious and Starking (Red Delicious), both of which are yellow-tissued varieties, and the McIntosh, a white-tissued variety. The apples were obtained from one of the chief fruit districts of Washington and were kept in a cool basement at a temperature of 40–45° F., this condition being comparable to good common storage. The apples averaged three and one-half months in storage at the time of feeding. They were taken from storage weekly and kept in an electric refrigerator at a maintained temperature of 40°F. for the daily feedings. They were peeled and chopped to insure uniform sampling, weighed quickly, and fed before there was appreciable evidence of brown discoloration from oxidation. These feedings were given to the rats daily six days per week throughout the experimental period.

A preliminary study was conducted in 1930–31 on four feeding levels of apple. These levels were 0.5, 0.75, 1.0, and 1.5 gm. per rat per day, the rats being fed for a 56-day period. The following year the study was repeated with all animals placed on the 1.5-gm. feeding level. The preliminary study showed that the three lower levels of apples were below the amount required to produce the unit weight gain for vitamin A. A 35-day

experimental period was employed in 1931–32. According to the U. S. Pharmacopoeia method (9), the unit for vitamin A is that amount of food which when fed to a test rat will induce a gain in weight of 10 to 20 gm. (an average of 3 gm. weekly) in a 35-day experimental period.

All animals were weighed weekly. The negative control groups received only the basal ration, while the positive control groups were given the basal ration into which was incorporated 10 per cent butter fat. Post-mortem examinations were made at the close of the experimental periods following the methods of Sherman and Munsell (8).

Experimental Data

The 45 rats used in the 1931–32 study averaged 44 gm. in weight when placed on the vitamin A-free basal ration for depletion of vitamin A reserves. The depletion period was approximately five weeks in length and the animals averaged 113 gm. when they were placed in individual cages at the time the apple feedings were initiated. The data of Table I show the

Table I

Rat Growth Records Comparing Apples with Yellow and with White Tissue as a Source of Vitamin A. (1931–32)

(35-day experimental period)

Supplement to basal diet	Amount fed daily, six days per week	Number of rats	Average weights		Average time of survival	Average loss or gain per week
			Initial	Final		
	gm.		gm.	gm.	days	gm.
Starking (Red Delicious) (yellow tissue)	1.5	9	113	125	35	+2.4
Golden Delicious (yellow tissue)	1.5	8	88	110	35	+4.4
McIntosh (white tissue)	1.5	8	107	132	35	+5.0
Positive control 10% butter fat	—	10	116	181	35	+13.0
Negative control (no test food)	—	10	112	86	19	−9.5

weight responses of the rats fed 1.5 gm. of Starking (Red Delicious), Golden Delicious, and McIntosh apples, together with those of the positive

and negative control groups. In the groups receiving apple feedings, the average weekly weight-gain was highest for the McIntosh, with Golden Delicious second, and the Starking (Red Delicious) third, though these differences were only slight. The negative control group showed marked evidence of infection, while the positive group was apparently protected in every respect.

Conclusions

The results of this study show there is practically no difference in the vitamin A content of the varieties of apples tested. Since the McIntosh, an apple with white tissue, shows a vitamin A potency at least equal to two yellow-meated varieties (Red Delicious and Golden Delicious), it would appear that the presence of yellow pigment in the apple is not related to vitamin A potency. The unit for vitamin A, as determined for these varieties is near the 1.5 gm. level. Munsell (10) has summarized her studies on the vitamin A value of certain foods and has reported the vitamin A unit for orange juice (Florida) to be 1.5 gm. and for apples (raw, cold storage) to be 2 gm. The three varieties of apples tested in the present study were richer in vitamin A than Munsell has indicated and it is clearly shown that they are equal to orange juice in vitamin A potency and compare favorably with other fruits as a source of vitamin A.

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EDITORIAL REVIEW

THE CHEMICAL NATURE OF ENZYMES

TWO of the most important problems in biological chemistry are the chemical nature of enzymes and their mode of action. These questions are as difficult as they are important. The purification of enzymes is an extremely troublesome task, owing to their great instability, their low concentration in plant and animal tissues, and their colloidal nature. Then, too the enzyme is markedly affected by changes in temperature, differences in pH, and by various impurities which may be present. Much of the earlier work with enzymes is now known to be of little value because before the time of Sörensen (1) the significance and effects of pH were not understood.

In their work with urease Sumner and Hand (2) found it unsafe to employ ordinary distilled water, for this usually contains traces of heavy metals and these poison the enzyme. Many of the freakish actions which have been observed with urease have been due to the use of impure distilled water, For example, the enzyme has been observed to wax and wane in activity, or it has been found to require the presence of some co-enzyme (3, 4), or activator (5–11) before exhibiting its full activity. Such substances as proteins, amino acids, or hydrocyanic acid have been described in the literature as functioning as auxo-substances, activators, or promotors, and all the while the observed effects have been due simply to a poisoning of the enzyme by heavy metals which was eliminated by the substance added (2, 12).

To just what extent this is the true explanation of the action of activators on other enzymes it is still too early to say. We have the interesting example of the vegetable protease papain, which digests proteins best after treatment with hydrocyanic acid, hydrogen sulfide, cysteine, or glutathione. According to Willstätter and Grassman (13) these substances function as activators; according to Krebs (14) they act by removing ions of heavy metals.

Enzymes have been known for about one-hundred years and until the extensive researches of the Willstätter school, which began about 1918, the enzymes had been regarded by most persons as proteins. There is con-

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siderable justification for this viewpoint since enzymes have many properties similar to those of proteins. A comparison is given below.

ENZYME	PROTEIN
colloidal nature	colloidal nature
soluble in water	soluble in water
inactivated at 60–80°C.	denatured at 60–80°C.
inactivated by acid and alkali	denatured by acid and alkali
inactivated by long contact with distilled water	denatured by long contact with distilled water
inactivation reversible in some instances	denaturation reversible in some instances
reversibly inactivated by heavy metals	reversibly precipitated by heavy metals
molecular weights above 20,000	molecular weights above 20,000

However, as the result of their very extensive researches, Willstätter and his students have taken the stand that enzymes are not of protein nature and that their chemical composition is entirely unknown. Willstätter (15, 16) has claimed that the enzyme exists adsorbed to some unspecific colloidal carrier.

The Willstätter school has purified enzymes for the most part by adsorption, making use of such adsorbents as kaolin and various preparations of aluminum hydroxide (17). As stated by Willstätter and Waldschmidt-Leitz (18): "There exists but a single method for the isolation of enzymes which is versatile, adaptable, and capable of development, namely the employment of adsorption processes based upon small affinity relations, or upon residual affinities." In many instances a considerable degree of purification has been achieved by use of adsorption methods, but in no case has any enzyme been obtained in condition of purity sufficient for any conclusion to be drawn regarding its chemical composition. Thus, yeast saccharase has been concentrated some 3000 to 4000 fold by adsorption and precipitation (16); animal lipase has been concentrated about 250 fold (19); liver esterase 128 fold (20); and castor oil lipase 100 fold (21). The greatest degree of concentration was that accomplished with the enzyme peroxydase, which Willstätter and his collaborators (22, 23) concentrated 20,000 fold. Recently Kuhn, Hand and Florkin (24) repeated this work and claim to have shown that, contrary to the conclusion of Willstätter, peroxydase contains iron as an essential constituent.

Willstätter (25) states that he obtained saccharase free, or almost free, from carbohydrates, phosphorus compounds, and proteins. "*Das Ergebnis war, dass sich die Saccharase von chemisch definierbaren hochmolekularen Stoffen, wie Kohlenhydraten, Phosphorverbindungen und Proteinsubstanzen, ohne Einbüsse an Aktivität, sogar an Beständigkeit, gänzlich oder fast ganz*

befreien liess." However, the highly purified saccharase contained from 4 to 10 per cent of nitrogen. One wonders if this could have been protein nitrogen. Willstätter (26) found some preparations of saccharase to be negative to the Millon and the ninhydrin tests. It is true, nevertheless, that the Millon reagent is not a delicate reagent for proteins which are poor in tyrosin. Besides, in testing for an unknown chemical substance one is always confronted with the possibility that the substance may be present in concentration too small to respond.

As a result of domination by Willstätter many biological chemists have come to regard enzymes as insusceptible of isolation. However, in 1926 Sumner (27) isolated from the jack bean a crystallizable globulin which he stated to be identical with the enzyme urease. Four years later Northrop (28) starting from high-grade commercial pepsin preparations obtained crystals which he believes to be identical with pepsin. Somewhat later Northrop and Kunitz (29) were able to obtain a crystalline protein possessing a high tryptic activity, whilst Caldwell, Booher and Sherman (30) reported that they had obtained from pancreatin a crystallizable protein which exerted high amylase activity. It is yet too early to discuss the incomplete evidence regarding crystalline trypsin and crystalline amylase, but for the isolation of urease (27, 31–37) and pepsin (28, 38, 39) in the form of crystallized globulins the experimental work is now both complete and convincing.

Urease crystals are obtained in an extremely simple manner (27). One mixes jack bean meal with 32 per cent acetone and filters in an ice-chest. The next day the filtrate is found to contain the crystals, which can be centrifuged off. If one employs jack bean meal rich in urease the amount of concentration is about 730 fold. Using meal of poor quality the purification possible is 1400 fold. Naturally the number of times that a substance can be purified is limited by the original concentration and the very fact that urease crystallizes directly from extracts of jack bean meal indicates a high concentration in the meal. The concentration of urease in the jack bean ranges from 0.07 to 0.15 per cent. If the urease in the soy bean is the same substance then the soy bean contains 0.01 per cent. When purified by recrystallizing from 32 per cent acetone, or from 30 per cent alcohol, the urease activity of the crystals eventually increased to a value of about 133,000 units per gram, and this figure has not been exceeded. It is of interest to note that Waldschmidt-Leitz (40) tried to purify urease by adsorption, using jack bean meal rich in urease, and that the most active material obtained had only 25,000 units per gram.

Many erroneous statements regarding crystalline urease have appeared in scientific journals and monographs. For example, the crystals have been described as "sphero-crystals" and have been said to be prepared by "precipitation with acetone." Haldane and Stern (41), in their recent text book, figure that crystalline urease of 130,000 units per gram would decompose one-fifth of its weight of urea per second. Since a urease unit has been defined as that amount of enzyme which will produce 1 mg. of ammonia nitrogen in 5 minutes at 20°C. one can reckon that 1 gm. of urease would split 928 mg. of urea per second, or practically its own weight. Under optimum conditions crystalline urease is somewhat more active than this. Again, they state that urease crystals obtained from jack bean meal poor in urease possess about one-half of the activity of crystals obtained from the best meal, but fail to mention the fact that the impure crystals can be raised in activity by recrystallization. They doubt whether crystalline urease should be classified as a globulin since it is soluble in distilled water. However, urease is not soluble in water at its isoelectric point unless salt is present. This property, together with its ability to coagulate when heated, places it among the globulins.

Certain enzyme chemists have attempted to draw conclusions regarding the purity of different enzymes by making comparisons of the velocities of substrate decomposition caused at a given temperature by a unit weight of enzyme. On this basis Northrop's crystalline pepsin is less active than crystalline urease and the urease is far less active than the admittedly impure peroxydase of Kuhn, Hand, and Florkin (24). However, there is no reason to suppose it possible to compare two enzymes catalyzing totally different chemical reactions; nor is there any *a priori* reason to expect all enzymes to be equally efficient as catalysts.

The method for preparing crystalline pepsin (28) is a little more complicated than that for preparing crystalline urease. Parke Davis 1–10,000 pepsin is dissolved in water, acidified with sulfuric acid, and salted out with magnesium sulfate. The precipitate is dissolved by the cautious addition of alkali and then reprecipitated by acid. This precipitate is dissolved by partly neutralizing and warming. Upon cooling from 45°C. pepsin crystals form. These crystals are about six times more active than the original material. The crystals are globulin in nature and have a constant activity that is not changed materially during seven recrystallizations. When a solution of crystals is heated, or treated with alkali, the inactivation of the pepsin proceeds at the same rate as the denaturation of the protein. Recently Northrop (38) has inactivated the pepsin completely

by addition of alkali and then has partly reactivated the enzyme by bringing the reaction to a faint acidity, as was done years ago with impure pepsin by Pawlow and Parastschuk (42). Next, Northrop fractionated and concentrated the active portion and obtained pepsin crystals possessing the same activity and crystalline habit as the original crystals.

Holter (43) has adsorbed crystalline pepsin upon aluminum hydroxide and claims to have fractionated it into two portions possessing different gelatin liquefying power. In view of the discovery by Northrop (44) that pepsin preparations contain a special gelatin-liquefying enzyme, Holter's observation does not appear to indicate that the crystalline pepsin is necessarily highly contaminated with foreign material. Northrop says: "This gelatinase is present in very small quantities in the original material and is completely removed from the crystalline pepsin only with considerable difficulty."

In 1885 Sundberg (45) obtained highly active pepsin solutions which gave no test for protein. His procedure was to extract the gastric mucosa of calf stomach with saturated sodium chloride for two or three days. The filtrate was dialysed against acidified water and then allowed to autolyse for one or two weeks. The pepsin was adsorbed upon calcium phosphate and the precipitate then dissolved in 5 per cent hydrochloric acid and dialysed. The pepsin thus obtained was negative to a large number of protein reagents. However, Sundberg states that the addition of absolute alcohol caused a white precipitate to form. This precipitate contained nitrogen and when heated upon platinum foil *gave a rather strong odor of burnt horn.* "*Beim Erhitzen auf einem Platinblecke wurde nämlich ein ziemlich starker Geruch nach gebrannten Horn wahrgenommen.*" What better test for protein or protein degradation products could one ask?

Willstätter and Rohdewald (46) have repeated Sundberg's work and have obtained pepsin solutions which even before adsorption upon calcium phosphate gave no Millon, xanthoproteic, or ferrocyanic acid tests. They have compared the activity of this pepsin with that of Northrop's crystalline pepsin, but, unfortunately, have employed pepsin crystals which had been dehydrated with acetone and which must have become partly inactivated. Considered as evidence against the protein nature of Northrop's crystalline pepsin their results are unconvincing.

Waldschmidt-Leitz (47) has taken up the attack against crystalline enzymes and has published a paper in which he states that he has obtained pancreatic amylase entirely free from protein. In collaboration with Steigerwaldt (40) he has published an article in which he shows that crys-

talline urease is not inactivated by trypsin, as would be expected if urease were a protein, and that after 48 hours incubation with trypsin at pH 7.0 and 30°C. the protein material comprising the crystals has become so far digested that a hardly perceptible precipitate is given upon adding sulfosalicylic acid. Since the urease is not inactivated by trypsin, whilst the protein is digested, he argues that the identity of urease with the urease crystals can be no longer maintained. The results of this paper were soon accepted by Willstätter (48).

At first sight the evidence looks convincing, but it should be noticed that Waldschmidt-Leitz and Steigerwaldt employed urease in a concentration of 1 part in 67,000, and that at this concentration urease gives hardly any visible precipitate with sulfosalicylic acid even before adding trypsin. On the other hand, as noted by Sumner and Kirk (36), trypsin itself gives such a heavy precipitate with sulfosalicylic acid that one is entirely prevented from observing the precipitate due to urease. Sumner and Kirk were able to detect no hydrolysis of crystalline urease by trypsin when they weighed the protein precipitable with sulfosalicylic acid. Furthermore, by making use of the precipitin test with immune rabbit serum (antiurease) they have shown that crystalline urease diluted 67,000 times is not appreciably affected by trypsin.

In a second paper Waldschmidt-Leitz and Steigerwaldt (49) have asserted that the precipitin reaction with antiurease is not a test for protein, but that it is a test for the enzyme itself. They have carried out new digestions, using purified trypsin and allowing it to act upon crystalline urease for longer periods of time. They find that the urease activity decreases less rapidly than the amount of protein present when the latter is estimated nephelometrically. Hence, they again state that there is no connection between the urease crystals and the enzyme, except that the crystalline protein serves as an especially suitable carrier.

Sumner, Kirk, and Howell (50) deny that antiurease precipitates only the urease and not the crystalline protein. They have investigated the effect upon crystalline urease of pepsin and papain-H_2S at pH 4.3 and find that while urease is slowly inactivated at this pH the presence of pepsin or papain increases the rate of inactivation many times. Under optimal conditions the inactivation of urease by active pepsin was found to be more than 20 times the rate of inactivation in the presence of boiled pepsin. Since pepsin in dilute solution gave no precipitate with dinitro-salicylic acid it was possible to follow the hydrolysis of urease turbidimetrically and to compare the rate of hydrolysis with the rate of inactivation. These two

have been found to be identical. The authors declare, therefore, that since urease is inactivated and simultaneously digested by pepsin and papain, the results of Waldschmidt-Leitz and Steigerwaldt, obtained by digesting urease with trypsin, cannot be used as an argument that urease is not a protein.

The controversy over the protein nature of urease reminds one of the similar dispute as to whether ricin, abrin, and other vegetable toxins were, or were not, proteins (51). Certain investigators (52) stated that the toxic properties were not diminished by incubation with trypsin and papain, while the digested material was found in some substances to be entirely free from protein. However, further investigation has shown ricin to be destroyed by pepsin (53) and by trypsin (54), although extraordinarily resistant to the latter enzyme, and the rate of its hydrolysis by trypsin has been shown to be identical with its rate of detoxication (55). At the present time the protein nature of the toxalbumins has found general acceptance, notwithstanding the fact that these substances, unlike urease and pepsin, have never been obtained in crystalline condition.

It would be of value and of interest to possess more data upon the effects of proteolytic enzymes on enzyme stability. However, the resistance of an enzyme to trypsin cannot be taken as final proof that the enzyme is not a protein. Nor, indeed, can the finding that urease and pepsin are proteins be taken to mean that all enzymes are proteins. Northrop (39) has attempted to digest his crystalline pepsin at pH 6.0 by both trypsin and papain. He found that the pepsin was not attacked, but instead the trypsin was rapidly inactivated, while the papain was slowly inactivated.

The question of the protein nature of urease is remotely related to the question as to the homogeneity of crystalline insulin. Dingemanse (56) adsorbed insulin on "supranorit" and then extracted with 85 per cent phenol. The amorphous product was four times more active than crystalline insulin. Because of this finding there has been some doubt as to whether crystallized insulin is identical with the hormone itself (48). Jensen (57), who believes in the identity, points out that: "Crystalline insulin has been prepared from the islet tissue of certain fishes and from pig and sheep pancreas. Insulin crystals from these various sources all possess the same activity, approximately 24 units per milligram, and all have the same sulfur content, 3.2 per cent." Moreover, the results of Dingemanse could not be repeated by du Vigneaud, Geiling, and Eddy (58), nor by Jensen and DeLawder (59). Nevertheless they are confirmed by the work of Dirscherl (60).

If enzymes are proteins they should act as antigens when injected into animals and one should be able to obtain antibodies of the type known as antienzymes from the animal's serum. The literature on enzymes contains many references to antienzyme formation, but most of the evidence submitted is far from convincing. Kirk and Sumner (37) have succeeded in obtaining antiurease by injecting crystalline urease into rabbits over long periods of time. Urease is very poisonous to mammals, for it converts their urea into ammonium carbonate and this causes death by alkalosis. As little as 0.15 mg. of crystalline urease is lethal for the average rabbit, but by using smaller doses it is possible to immunize. A highly immunized rabbit can tolerate in one day 400 lethal doses. The normal rabbit or guinea pig can be protected against urease by giving an injection of serum taken from an immune rabbit. The hen is not poisoned by injecting urease, as S. F. Howell (61) found, for the hen has only about 2 mg. of urea per 100 cc. of blood. However, injection of urease into the hen brings about the formation of antiurease just the same.

The serum of immunized animals contains antiurease. This substance inhibits the hydrolysis of urea by urease. When urease is added to immune serum a precipitate is formed. The reaction is so sensitive that urease at a final dilution of 600,000 times still gives a detectable haziness. This is the test which was alluded to when it was stated that urease continues to give the precipitin test even after long incubation with trypsin. It is of interest to note that soy bean urease is precipitated also by jack bean antiurease, from which it appears that soy bean urease is either identical with jack bean urease, or else closely related (62).

It is a very easy matter to purify antiurease (37). One adds crystalline urease to immune serum and, after keeping at 38°C. for 30 minutes, centrifuges off the precipitate of urease-antiurease. The precipitate is washed twice with dilute salt solution and is then dissolved in dilute hydrochloric acid. The acid denatures the urease, but has no effect on the antiurease. Upon neutralizing, the denatured urease precipitates, leaving the antiurease in solution. The recovery of antiurease is sometimes as high as 95 per cent.

Antiurease is probably a globulin. It is precipitated by dialysis and is inactivated by long dialysis. It is not affected by long incubation with trypsin at pH 7.0, but is rapidly inactivated by papain and papain-HCN at pH 5.0 and by pepsin at pH 4.3. Preliminary experiments indicate that antiurease unites stoichiometrically with crystalline urease.

Summary

It is the author's conviction that the proof of the identity of urease and pepsin with crystalline urease and crystalline pepsin is complete and that any hypothesis to the contrary would necessitate highly improbable assumptions. Adsorption methods have been fully tried out and have been found inadequate for the isolation of enzymes. Evidence intended to disprove the identity of urease crystals with the enzyme itself has either failed to bring proof, or has actually led to further investigations resulting in confirmation of earlier conclusions. At least two proteins have been prepared in condition of high purity, and have been demonstrated to possess intense enzymic activity. The activity has been shown to remain constant after repeated recrystallization and to be diminished by the action of agents which bring about denaturation, or digestion, at the same rate that the crystalline proteins are denatured, or digested. It would appear to the author that only a detailed investigation of the chemically active groups present in urease and pepsin can be expected to shed further light on their chemical nature.

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